UNIVERSIDAD DE CÓRDOBA

Programa de Doctorado en Biociencias y Ciencias Agroalimentarias

Bioaccesibilidad de elementos inorgánicos en leguminosas y potitos ecológicos de base vegetal

Bioaccessibility of inorganic elements in legumes and vegetable-based organic weaning food

Tesis doctoral presentada por

Ana María Ramírez Ojeda

DIRECTOR:
Dr. D. Fernando Cámara Martos

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AUTOR: *Ana María Ramírez Ojeda*

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Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

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TÍTULO DE LA TESIS: Bioaccesibilidad de elementos inorgánicos en leguminosas y potitos ecológicos de base vegetal

DOCTORANDA: Dña. Ana María Ramírez Ojeda

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(Se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

La doctoranda ha desarrollado su tesis doctoral bajo mi supervisión directa, investigando el contenido de elementos inorgánicos en leguminosas y potitos ecológicos de base vegetal. También ha realizado una valoración de la bioaccesibilidad de estos elementos inorgánicos en las muestras estudiadas, así como la influencia de otros componentes nutricionales (proteínas, grasa y fibra dietética) en dicha bioaccesibilidad. Los resultados obtenidos han servido para realizar una valoración nutricional de estos grupos de alimentos.

La tesis ha dado lugar a las siguientes publicaciones en forma de capítulo de libro o artículos científicos publicados en revistas indexadas en JCR:

- **Ramírez – Ojeda A.** Peas and Lentils (2016). Encyclopedia of Food and Health 1st Edition, 238 – 288. Elsevier: Academic Press.
- **Ramírez – Ojeda A.,** Moreno – Rojas R., Cámara –Martos F. **2018.** Mineral and trace element content in legumes (lentils, chickpeas and beans): Bioaccesibility and probabilistic assessment of the dietary intake *Journal of Food Composition and Analysis, 73,* 17 – 28. Food Science and Technology (Q1; 32/133).
- **Ramírez – Ojeda A.,** Moreno – Rojas R., Sevillano – Morales J., Cámara – Martos F. **2017.** Influence of dietary components on minerals and trace elements bioaccessible fraction in organic weaning food: a probabilistic assessment. *European Food Research and Technology, 243,* 639 – 650. Food Science and Technology (Q2; 61/133).

- Cámara –Martos F, **Ramírez – Ojeda A.,** Jiménez – Mangas M., Sevillano – Morales J., Moreno – Rojas R. **2019.** Selenium and cadmium in bioaccessible fraction of organic weaning food: Risk assessment and influence of dietary components. *Journal of Trace Elements in Medicine and Biology, 56,* 116 – 123. Biochemistry & Molecular Biology (Q2; 146/299).

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 14 de enero de 2021

Firma del director

Fdo.: Fernando Cámara Martos

INFORME DE ÍNDICES DE IMPACTO

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Influence of dietary components on minerals and trace elements bioaccessible fraction in organic weaning food: a probabilistic assessment (2017). ***European Food Research and Technology,*** *243,* 639 – 650	Food Science & Technology 1.919	Q2 (61/133)
Selenium and cadmium in bioaccessible fraction of organic weaning food: Risk assessment and influence of dietary components (2019). ***Journal of Trace Elements in Medicine and Biology,*** *56,* 116 – 123	Biochemistry & Molecular Biology 2.895	Q2 (146/299)

El éxito no es definitivo,
el fracaso no es fatal: lo que
realmente cuenta
es el valor para continuar.

Winston Churchill

A mi familia,

por su apoyo incondicional

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AC	Alimentación complementaria
AEP	Asociación Española de Pediatría
AF	Aflatoxinas
AOAC	Association of Official Agricultural Chemists
DON	Deoxynivalenol
DRI	Dietary References Intakes
EFSA	European Food Safety Authority
EU	European Union
EUROSTAT	Oficina Estadística de la Unión Europea
FAO	Food And Agriculture Organization Of The United Nations
FAOSTAT	The Food and Agriculture Organization Corporate Statistical Database
FEN	Fundación Española de la Nutrición
FESNAD	Federación Española de Sociedades de Nutrición, Alimentación y Dietética
IFOAM	International Federation of Organic Agriculture Movements
IYP	International Year of Pulses
OMS	Organización Mundial de la Salud
ONU	Organización de las Naciones Unidas
OPS	Organización Panamericana de la Salud
PTWI	Permitted tolerable weekly intake
SEGHNP	Sociedad Española de Gastroenterología Hepatología y Nutrición Pediátrica
USDA	United States Department of Agriculture
WHO	World Health Organization
ZEN	Zearalenone

RESUMEN

La infancia es un periodo durante el cuál las necesidades nutricionales se ven aumentadas. Por otro lado, cabe destacar que, durante las últimas décadas, se ha producido un aumento del consumo de productos ecológicos cuya tendencia se ha trasladado al caso de alimentos infantiles. Sin embargo, no existe la suficiente información para determinar si dichos productos proporcionan los adecuados requerimientos nutricionales del niño. También debemos señalar que el año 2016 fue proclamado como el año de las legumbres, con el objetivo de aumentar la conciencia sobre los beneficios nutricionales de dichas legumbres (alcanzar la seguridad alimentaria, combatir la malnutrición, reducir la pobreza, etc). Por otro lado, debe recalcarse que, para establecer los requerimientos nutricionales, debemos determinar la concentración de mineral soluble presente en el lumen intestinal y susceptible de ser absorbida, así como la influencia de ciertos factores sobre la bioaccesibilidad. Esta información proporcionará el consumo real del nutriente y normalmente es obtenida usando métodos *in vitro*.

Con todo esto, el propósito de la presente tesis doctoral fue abordar el análisis tanto del contenido total y bioaccesible (solubilidad y dializabilidad) de elementos traza presentes en potitos categorizados con el atributo "ecológico", así como también en tres variedades de legumbres ampliamente consumidas en la población española vendidas en dos formatos, con el objetivo de evaluarlos nutricionalmente. Para ello, se determinó el contenido de algunos elementos inorgánicos (Fe, Zn, Cu, Mn, Ca, Mg, Se y Cd) así como también se realizaron ensayos *in vitro* de bioaccesibilidad (se utiliza la solubilidad y la dializabilidad como criterios evaluadores de la biodisponibilidad mineral). Asimismo, también es estudiada la influencia de determinados componentes alimentarios (fibra, grasa y proteínas) en la bioaccesibilidad de estos elementos traza. Posteriormente se evaluó la contribución de estos dos grupos de productos a la Ingesta Dietética de Referencia (IDR). La obtención de estos datos nos permitiría establecer recomendaciones para promocionar el consumo de estos productos.

Palabras clave: alimentos infantiles, productos ecológicos, legumbres, bioaccesibilidad, elementos inorgánicos.

ABSTRACT

Childhood is a period during which nutritional needs are increased. In recent decades, as should be noted, there has been an increase in the consumption of organic products. This trend has also /been found in the case of baby foods. However, there is not enough information to determine if these products provide the adequate nutritional requirements of the child. It should also note that 2016 was proclaimed as the year of legumes, with the aim of increasing awareness about the nutritional benefits of legumes (achieving food security, combating malnutrition, reducing poverty, etc.). Also, it should be emphasized that, to establish nutritional requirements, the concentration of soluble mineral present in the intestinal lumen and its capable of being absorbed must be determined, as well as the influence of certain factors on bioaccessibility. This information will provide the actual intake of the nutrient and is normally obtained using *in vitro* methods.

Taking into account the above, the purpose of this doctoral thesis is to address the analysis of total and bioaccessible content (solubility and dialyzability) of trace elements present in baby food categorized with the "ecological" attribute, as well as in three varieties of legumes widely consumed in the Spanish population, sold in two formats. The aim was to evaluate them nutritionally. To this end, the content of some inorganic elements (Fe, Zn, Cu, Mn, Ca, Mg, Se and Cd) was determined as well as in vitro bioaccessibility tests being carried out (solubility and dialyzability were used as evaluation criteria of mineral bioavailability). Likewise, the influence of certain food components (fiber, fat and proteins) on the bioaccessibility of these trace elements has also studied. Subsequently, the contribution of these two groups of products to the Dietary Reference Intake (RDI) was evaluated. Obtaining this data would allow us to establish recommendations to promote the consumption of these products.

Keywords: baby food, organic products, legumes, bioaccessibility, inorganic elements.

Introducción

1. INTRODUCCIÓN

1.1. ALIMENTACIÓN INFANTIL

Introducción

La importancia de una adecuada nutrición durante la infancia reside no solo en su papel para garantizar un correcto crecimiento y desarrollo del niño, sino que una mala nutrición infantil, está asociada con un aumento en el riesgo de contraer enfermedades en la edad adulta (OMS, 2010; Gregory & Walker, 2013; Mir-Marqués et al., 2015). Dentro de la nutrición infantil, la lactancia materna es muy importante para el desarrollo futuro del individuo, ya que ha demostrado su capacidad para reducir el riesgo de padecer enfermedades en etapas posteriores de la vida; entre ellas, se asocia a una menor probabilidad de presentar sobrepeso y obesidad (Baker et al., 2004; Grummer-Strawn & Mei, 2004; Gruszfeld & Socha, 2013; Sandoval Jurado et al., 2016) (Tabla 1).

Tabla 1: Beneficios de la lactancia materna (Maldonado Lozano, 2018)

Beneficios a corto plazo	Beneficios a largo plazo
- Protección frente a infecciones gastrointestinales y respiratorias y alergias	- Reducción de la incidencia de obesidad y diabetes - Menores niveles de colesterol y presión arterial - Mayor puntuación en test de inteligencia

Por otro lado, la lactancia materna está considerada para un neonato la mejor forma de alimentación natural, exclusiva hasta los 6 meses de edad, ya que cubre todas sus necesidades nutritivas (WHO, 2008; OMS, 2013). A partir de esta edad y hasta los 2 años, aunque se puede seguir utilizando la leche materna como alimento, es necesaria una alimentación complementaria (AC), consistente en incorporar otros alimentos en estado semisólido. Así, la OMS (2015) afirma que es preciso añadir otros alimentos a la dieta de los infantes cuando la lactancia natural ya no basta para satisfacer las necesidades nutricionales del niño. Por tanto, este proceso, podría definirse como un período durante el cual un bebé depende cada vez menos de la leche materna y se acostumbra lentamente a consumir alimentos para adultos.

Objetivos de la AC

Entre los objetivos que persigue la AC podemos destacar los siguientes (OPS/OMS, 2010) (Figura 1):

- Prevenir las deficiencias de nutrientes debidas a una disociación entre la disponibilidad de estos en la leche materna y los requerimientos del niño en función de su crecimiento, con especial énfasis en el hierro y zinc.
- Conducir al niño a aceptar nuevos alimentos, inculcando el gusto por los distintos sabores y texturas.
- Fomentar hábitos alimentarios saludables y prevenir factores de riesgo.
- Desarrollar habilidades que permitan una transición fluida en el niño entre la dependencia total de las madres para alimentarse, a la alimentación por sí mismo.

En definitiva, los alimentos complementarios, juegan un papel importante en la dieta del bebé, ya que constituyen la principal fuente de energía para niños de entre 6 y 12 meses (Pardío-López, 2012) y son utilizados con el objetivo de promover una mejor nutrición, desarrollo y salud (Melø et al., 2008; MacLean et al., 2010; Ljung et al., 2011; Pehrsson et al., 2014).

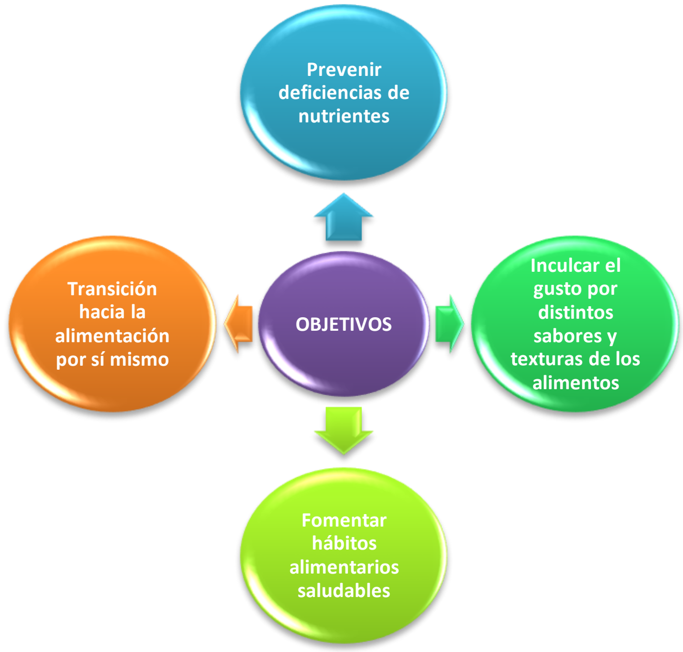


Figura 1. Objetivos de la AC

¿Cuándo y por qué empezar la AC?

Las recomendaciones de la Sociedad Europea de Gastroenterología, Hepatología y Nutrición Pediátrica (SEGHNP) del 2008, señalan que el inicio de la AC no debe ser ni antes de las 17 semanas ni después de las 26 semanas de vida, tanto en niños amamantados, como en los que reciben fórmulas lácteas infantiles o lactancia mixta (Agostoni et al., 2008). Así, la introducción temprana de alimentos distintos a la leche materna podría tener riesgos tanto a corto plazo: incremento de infecciones gastrointestinales (Fewtrell et al., 2017), desnutrición como causa de la disminución del consumo de leche materna e introducción de alimentos de baja densidad energética (Kramer & Kakuma, 2012), anemia debido a deficiencias de hierro, deficiencias de zinc, incremento de enfermedades respiratorias, daño renal… (Romero-Velarde et al., 2016), como también podemos encontrar diversos riesgos a largo plazo: alergias, aumento de la adiposidad (Solomons, 2008) o predisposición a desarrollar hipertensión y obesidad (Fall et al., 2011). Sin embargo, un retraso en dicho inicio, también podría provocar efectos negativos: rechazo a alimentos sólidos, vómitos, atragantamientos (Bernard-Bonnin,

2006) y no cubrir las necesidades nutricionales (energía, proteínas, hierro, zinc) del lactante debido a que la lactancia materna exclusiva ya no es suficiente (WHO, 2005; Vásquez Garibay et al., 2012).

Por otro lado, en relación a esta transición hacia los alimentos sólidos, para su buena implementación, se debe resaltar que ésta varía no solo en función de la cultura, sino que debemos tener en cuenta múltiples factores como: necesidades individuales del niño (Fewtrell et al., 2017; Iñiguez León, 2017) edad, medio socioeconómico en el que se encuentra (Cuadros-Mendoza et al., 2017). Además, el inicio de la AC dependerá de factores anatómicos, fisiológicos, habilidades sociales y motoras adquiridas (Arvedson, 2006; Delaney & Arvedson, 2008) (Tabla 2) que influirán en la capacidad del niño para la digestión y absorción de los diferentes macro y micronutrientes presentes en la dieta. Estas habilidades, adquiridas conforme el niño va creciendo, permiten la modificación de su alimentación (Butte et al., 2004; Pardío-López, 2012).

Tabla 2: Habilidades sociales y motoras adquiridas según la edad (Romero-Velarde et al., 2016; Cuadros-Mendoza et al., 2017)

Edad (meses)	Habilidades sociales y motoras adquiridas
0-3	Succión, deglución, reflejo de extrusión de la lengua, y reflejo de búsqueda.
3-6	Posición sedente, reflejos de extensión protectora. Sostén cefálico. Aumento de la fuerza de succión. Movimientos laterales de la mandíbula. Desaparece reflujo de protrusión. Deglución voluntaria. Se lleva las manos a la boca.
6-9	Gateo, bipedestación y apoyo en cuatro puntos. Buen control muscular. Chupa la cuchara con los labios. Movimientos laterales con la lengua. Empuja la comida hacia los lados. Toma alimentos con las manos. Lleva objetos con la mano y lleva a la boca. Movimientos rotatorios masticatorios.
9-12	Extiende su interés por variedad en texturas y alimentos, es más independiente para comer aunque requiere apoyo, puede morder y masticar algunos alimentos, mejora la funcionalidad de los músculos de la cavidad oral, desarrollo de propiocepción con integración de funciones motoras y coordinación.

En definitiva, se debe asegurar una ingesta energética adecuada en los niños y dada la incapacidad de la leche materna y de las fórmulas lácteas infantiles de asegurar un aporte energético idóneo que garanticen el crecimiento y desarrollo apropiado del lactante, se hace necesaria la introducción de la AC (Romero-Velarde et al., 2016).

Cómo introducir los alimentos

La AC debe basarse en alimentos que habitualmente consume la familia (Cuadros-Mendoza et al., 2017), aunque siempre deberá considerarse que los alimentos que aportemos en la dieta del niño tienen que ser ricos en todos los micronutrientes y principios inmediatos (OPS/OMS, 2010; Romero-Velarde et al., 2016; Fewtrell et al., 2017). Entre ellos, se incluyen una amplia variedad de grupos como cereales, frutas, verduras, carne, pescado, etc, dando lugar a una amplia gama de productos para niños en el mercado. Sin embargo, es importante enfatizar que estos alimentos no deben sustituir completamente a la leche materna, sino que son un complemento (Melø et al., 2008; Ljung et al., 2011). Para la introducción de dichos alimentos debemos considerar una serie de factores como son los siguientes:

- **Consistencia:**

Los alimentos iniciales deben ser semisólidos y tener una consistencia y sabor suave. Si bien el continuar con alimentos semisólidos puede ahorrar tiempo, es importante que a medida que el niño crece, la consistencia y la variedad de los alimentos se incrementen de manera gradual adaptándose a sus requerimientos y habilidades (OPS/OMS, 2010; Cuadros-Mendoza et al., 2017).

Al inicio de los 6 meses, el lactante puede comer alimentos sólidos o semisólidos, en forma de puré o aplastados. Los primeros alimentos adecuados son los cereales. En muchas partes del mundo, el primer alimento que se introduce es un cereal. Así, en los países en desarrollo, lo más probable es que se aporte una papilla, mezclándose el cereal básico local con leche o agua, mientras que, en las culturas de Europa occidental, el primer alimento es un cereal infantil preparado comercialmente que contiene además vitaminas y minerales añadidos (Cuadros-Mendoza et al., 2017).

La textura de los alimentos administrados a partir de 6 meses de edad, debe progresar gradualmente de semisólidos a alimentos blandos. Se pueden dar cereales integrales o cereales de avena instantánea además del huevo bien cocido. Los alimentos adecuados para comer con los dedos incluyen frutas suaves y zanahoria cocida. A partir de los 10 meses, los alimentos como la carne se deben picar finamente. Las verduras cocidas solo necesitan ser picadas, y se pueden dar platos de pasta con forma pequeña. Finalmente, a los 12 meses, la mayoría de los niños pueden comer los mismos alimentos que consume el resto de la familia (Cuadros-Mendoza et al., 2017) (Tabla 3).

Tabla 3: Consistencia y alimentos a introducir según la edad

Edad (meses)	0-6	6-12	12-24	>3 años
Consistencia y alimentos	Líquida	Pures, alimentos machacados picados finos	Trocitos pequeños	
Leche materna				
Leche adaptada (en niños que no toman leche materna)				
Cereales, frutas, hortalizas, legumbres, huevos, carne, pescado, aceite de oliva				
Leche entera, yogur y queso tierno (pueden ofrecerse en pequeñas cantidades a partir de los 9 o 10 meses)				
Sólidos con riesgo de atragantamiento (frutos secos enteros, palomitas, granos de uva, manzana o zanahoria cruda)				Por encima de los 3 años
Alimentos superfluos (azúcar, miel, mermelada, cacao, chocolate, flanes, galletas, bollería, embutidos y charcutería)	Cuanto más tarde y en menor cantidad mejor (siempre a partir de los 12 meses)			

- **Cantidades:**

El número de comidas que un lactante o niño pequeño necesita, depende de:

- *La energía que el niño necesita y la densidad energética del alimento ofrecido*. La cantidad de alimentos que debemos aportar dependerá tanto de las kilocalorías que necesita el niño, como de la energía que aportan dichos alimentos; es decir, de la densidad energética del alimento ofrecido (número de kilocalorías por mililitro o por gramo). La leche materna contiene aproximadamente 0,7 kcal por mL, mientras que los alimentos complementarios son más variables (usualmente, contienen entre 0,6 – 1,0 kcal por gramo) (Romero-Velarde et al., 2016). La densidad de energía de los alimentos complementarios debe ser mayor que la de la leche materna (al menos 0,8 kcal por gramo). Si la densidad energética es menor, para llenar la brecha de energía se requiere un mayor volumen de alimentos (OPS/OMS, 2010). Generalmente, los alimentos más espesos o sólidos tienen mayor densidad de energía que los alimentos aguados o muy blandos (aproximadamente solo contienen 0,3 kcal por gramo). Por tanto, cuando el niño come alimentos espesos o sólidos, resulta más fácil cubrir los requerimientos de macro y micronutrientes con un menor aporte energético.

- *La cantidad de alimentos que el niño pueda comer en una comida*. A su vez, la cantidad de comida ingerida por un infante depende de la capacidad de su estómago (normalmente es de 30 mL por kg de peso). Un niño que pesa 8 kg tendrá una capacidad gástrica de 240 mL, durante una comida, por tanto, no podrá tomar más de esta cantidad. Sin embargo, esta capacidad aumenta de 30 mL a 80 mL durante el primer mes y a 120 mL por kg de peso en las seis semanas siguientes (PAHO/WHO, 2003; van Dijk et al., 2009). Por tanto, a medida que aumenta su capacidad gástrica, la cantidad de alimentos a ingerir también aumenta gradualmente. Así, el número de comidas que puede realizar el niño a lo largo del día puede empezar siendo de una al día, y aumentar hasta dos o tres comidas al día. Además de estas comidas podemos ofrecer colaciones o meriendas, procurando que sean nutritivas.

Debe tenerse en cuenta que ofrecer al niño una baja cantidad de comidas al día, no cubrirá los requerimientos energéticos diarios y un exceso de comidas diarias favorecerá el abandono precoz de la lactancia, incremento de peso y obesidad (OPS/OMS, 2010; (Romero-Velarde et al., 2016).

Preferencias alimentarias

Por otro lado, debe favorecerse el desarrollo de hábitos nutricionales saludables en el niño, utilizando para ello la introducción de nuevos alimentos con sabores y texturas diferentes (Maldonado Lozano, 2018). Algunos estudios sugieren que las experiencias con sabores y las preferencias alimentarias durante la infancia siguen hasta la niñez y la adolescencia (Iñiguez León, 2017). Las mejores prácticas en la alimentación infantil recomiendan alimentos no voluminosos y de sabor simple para ayudar a establecer el umbral de percepción del bebé para estos gustos a niveles más bajos de los que más adelante percibirá en su vida (Mennella et al., 2011; Ventura & Worobey, 2013; Campos Rivera & Lagunes, 2014).

Los primeros aprendizajes de sabores se establecen en el útero. La elección de la dieta por parte de la madre, cambia el sabor al líquido amniótico (Beauchamp & Mennella, 2011). Además, durante la lactancia, las preferencias alimentarias del niño también pueden variar. En función de los alimentos y bebidas que toma la madre, estos aportarán diferentes sabores a través de la leche materna (Maldonado Lozano, 2018).

Requerimientos nutricionales

Los requerimientos energéticos en el niño dependen de la edad, de la frecuencia de su ingesta y de la cantidad de leche materna consumida (Egli, 2001; Dewey & Brown, 2003; WHO/UNICEF, 2003). Sin embargo, debemos tener en cuenta que, dentro de unos patrones generales, se deben valorar las necesidades de cada niño individualmente, ya que, entre otros factores, también pueden influir la velocidad de crecimiento, el desarrollo neurológico, el grado de actividad y las necesidades basales (Gil Campos et al., 2017). Para estimar los requerimientos que deben ser aportados por la alimentación complementaria, se debe calcular la diferencia entre los requerimientos totales estimados para el grupo de edad y los aportados por la leche materna. Así, en lactantes amamantados, la energía que debe aportar la AC sería de 130 a 200 kcal/día (6 a 8 meses); de 300 a 310 kcal/día (9 a 11 meses); y de 550 a 580 kcal/día (12 a 23 meses) (WHO, 2001; Dewey & Adu-Afarwuah, 2008; OPS/OMS, 2010).

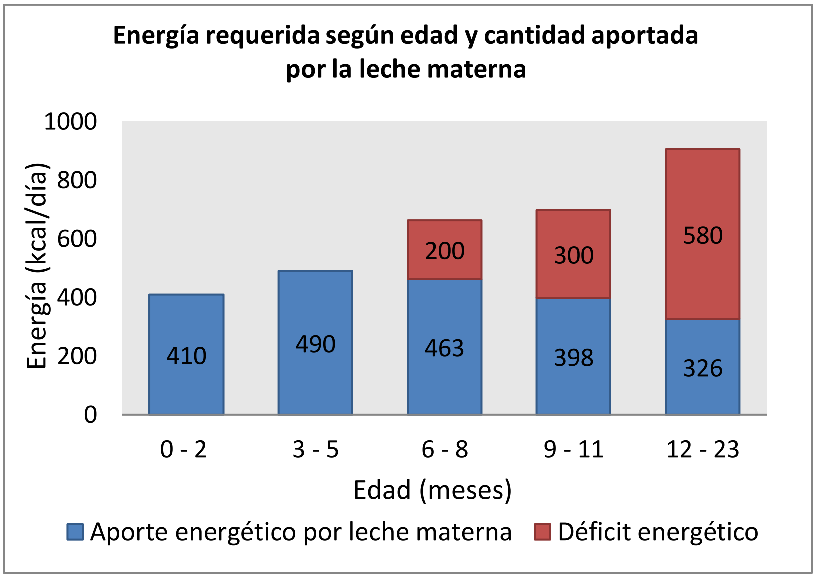


Figura 2: Energía requerida según edad (Kcal) y cantidad aportada por la leche materna y los alimentos complementarios (Cuadros-Mendoza et al., 2017)

Como puede ser observado en la gráfica, conforme el niño va creciendo existen mayores requerimientos energéticos, que empiezan a ser cubiertos en mayor medida por la AC, desplazando de este modo a la leche materna.

Hidratos de carbono y fibra

En relación a los hidratos de carbono, para niños hasta los 3 años, deben suponer un 40-55% del total de la ingesta energética. Esta ingesta se verá cubierta sin ningún tipo de problema durante la lactancia materna, aportado principalmente por la lactosa. Una vez iniciada la AC, empiezan a incorporarse otros hidratos de carbono, principalmente presentes en frutas como plátano, manzana, pera (posponiendo la introducción de algunas como melocotón, fresas, kiwi, por ser potencialmente alergénicas y verduras ricas en vitamina A y C como zanahorias, tomates, pimientos (Gil Hernández et al., 2006; OMS/OPS, 2010). Se recomienda que los hidratos de carbono aportados sean complejos, debiendo tener especial cuidado con la ingesta de azúcares simples, ya que pueden provocar ciertas enfermedades como sobrepeso y obesidad e incluso caries (SEGHNP-AEP, 2010). Estos azúcares simples no deben superar el 10% del total de calorías aportadas por los hidratos de carbono (Comité de Nutrición, 2001). No se debe tampoco añadir azúcar a las comidas y evitaremos los zumos de frutas y las bebidas azucaradas (Fewtrell et al., 2017).

Con relación a la fibra, hidratos de carbono no digeribles (solubles e insolubles) que alcanzan el intestino grueso sin ser degradados, existe cierta controversia en su recomendación (Gil Hernández et al., 2006). Por un lado, tiene efectos beneficiosos como su efecto sobre el vaciado gástrico y la saciedad. Así, diferentes estudios han observado el papel de la ingesta de fibra dietética durante la infancia para reducir el riesgo de desarrollar estreñimiento, obesidad, diabetes y enfermedades cardíacas (Institute of Medicine, 2005; Kranz et al., 2012; Dahl & Stewart, 2015). Sin embargo, también puede tener efectos negativos, ya que puede ser irritante del intestino o puede interferir en la absorción de micronutrientes por la presencia de otros compuestos como los fitatos (Hurrell et al., 2003; Frontela et al., 2009; Rasane et al., 2014).

Es por ello que no se han determinado los requerimientos de fibra para niños de 6 a 12 meses. En cualquier caso, la fibra debe ser introducida gradualmente a través de cereales, frutas y verduras y no debe superar la cantidad de 5g por día hacia el final del año de edad (Finn et al., 2019). A partir de los 2 años, la American Health Foundation estableció unas recomendaciones mínimas de fibra dietética empleando para ello la fórmula de cálculo de los requerimientos que se utiliza para los niños mayores, es decir, "edad + 5 g /día"(Gil Hernández et al., 2006). Sin embargo, en la actualidad, se ha establecido una ingesta adecuada para niños de edades comprendidas entre 1 y 3 años de 19 g por día, recomendaciones que han sido adoptadas tanto por el Institute of Medicine de EEUU como por la American Academy of Pedriatrics y la American Dietetetic Assocition (Finn et al., 2019; Megias Rangil & Salas-Salvadó, 2019).

Proteínas

Con respecto a los requerimientos de proteínas, a lo largo de los años, las recomendaciones de ingesta proteica han ido disminuyendo y actualmente se considera una ingesta adecuada para estas edades de 1,5-2 g de proteína/kg de peso y día (Directiva 2006/141/CE). Sin embargo, es importante no sobrepasar estas cantidades, puesto que una ingesta alta de proteínas durante los 2 primeros años de vida puede tener efectos sobre la salud a largo plazo (mayor porcentaje de obesidad infantil) (Gil Campos et al., 2017). Por tanto, resulta muy relevante evitar dietas hiperproteicas, que pueden incrementar el riesgo de sobrepeso y obesidad (Haschke et al., 2016).

Durante esta etapa, también son elevados los requerimientos de aminoácidos esenciales entre los que encontramos a isoleucina, leucina, lisina, valina, metionina, fenilalanina, treonina, y triptófano. También deben ser considerados como esenciales la histidina hasta los 6 meses y la cisteína en el recién nacido puesto que la capacidad para sintetizarlos es menor que sus requerimientos (Gil Campos 2005). Para cubrir éstos es importante aportar proteínas de alto valor biológico (como las contenidas en ciertos alimentos como carne, huevo o leche y sus derivados) (Comité de Nutrición, 2001); aunque con la edad, estos requerimientos van disminuyendo (SEGHNP-AEP, 2010).

Lípidos

Cabe destacar que las grasas son una fuente importante de energía en los primeros meses de vida, sin embargo, a partir de los 6 meses decrece su contribución a favor de los hidratos de carbono. La ingesta diaria de grasa en niños menores de dos años debe suponer un 30-45% de la ingesta energética diaria (Butte et al., 2004; SEGHNP-AEP, 2010; Romero-Velarde et al., 2016). Dicho porcentaje no debería ser superior puesto que no se aseguraría un correcto equilibrio con el consumo de otros alimentos que aporten proteínas o minerales entre otros nutrientes importantes que debemos garantizar (SEGHNP-AEP, 2010). El aporte de estas grasas nos permitirá, además, una correcta absorción de vitaminas liposolubles (OPS/OMS, 2010).

Por otro lado, debe garantizarse el correcto aporte de ácidos grasos esenciales como el ácido linoleico y linolénico, asegurándose que exista un buen equilibrio entre ambos (los dos compiten por las mismas enzimas en las reacciones de desaturación y elongación). Así, entre los 6 y los 12 meses, el ácido linoleico debe aportar un 4% de la ingesta energética y un 0,5% debe ser aportado por el ácido linolénico. También debe aportarse 100mg/día de ácido docosahexaenoico (Butte et al., 2004; EFSA, 2013; Fewtrell et al., 2017).

En la siguiente tabla (Tabla 4) se muestran los requerimientos de energía y principios inmediatos (Romero-Velarde et al., 2016); para diferentes grupos de edad durante los primeros meses de vida.

Tabla 4: Requerimientos nutricionales (Romero-Velarde et al., 2016)

Edad	Kcal/d	g Prot/kg/d	Proteínas (%)	Lípidos (%)	Glúcidos (%)
6-8 meses	641	1,5	6 - 8	40 - 60	32 - 54
9-11 meses	713	1,5	6 - 8	35 - 40	50 - 55
12-23 meses	906	1,1	10 - 14	35	55 - 60

Vitaminas

La utilización de suplementos vitamínicos suele ser innecesaria, puesto que gracias a una ingesta variada de alimentos que supone la introducción de la AC, hace que se vean cubiertas las necesidades de dichas vitaminas. Sin embargo, cabe destacar que existen situaciones en las que puede ser necesaria una cierta suplementación con algunos micronutrientes, como puede ser el caso de las vitaminas A y D.

La vitamina A, comprende todos los compuestos que poseen actividad biológica de retinol. Se almacena principalmente en el hígado e interviene en las funciones inmunes, así como en el crecimiento, diferenciación y proliferación celular (Comité de Nutrición, 2001). Los requerimientos diarios recomendados por vía oral se encuentran entre 1332 Unidades Internacionales (UI) (400 µg de retinol) para niños menores de 6 meses; 1665 UI (500 µg de retinol) para niños con edades comprendidas entre los 6 – 12 meses; y 999 UI (300 µg de retinol) para niños entre 1 – 3 años. Sin embargo, en países con un riesgo elevado de deficiencia, donde la carencia de vitamina A constituya un problema de salud pública, se recomienda administrar una dosis de vitamina A suplementaria que aseguren un correcto aporte. Así, la OMS, (2011) recomienda administrar un suplemento de vitamina A de 100.000 UI en una única vez para niños de entre 6 y 12 meses; y en niños mayores de 12 meses 200.000 UI cada 4-6 meses.

También, durante el primer año de vida se recomienda una suplementación oral diaria de 400 IU de vitamina D (10 µg/día) (Braegger et al., 2013). La vitamina D, puede provenir de la dieta o ser sintetizada en la piel por exposición al sol (Braegger et al., 2013). Interviene principalmente en el metabolismo del calcio y del fósforo (Chung et al., 2009) y es esencial en el mantenimiento de la salud ósea.

Por otro lado, la vitamina E posee capacidad antioxidante y contribuye principalmente a prevenir la propagación de radicales libres (Traber & Stevens, 2011). La Agencia Europea de Seguridad Alimentaria (EFSA) (2013) recomienda unos 5 mg/día desde los 6 - 12 meses y 6 mg/día hasta los 36 meses de edad. Su deficiencia es rara, ya que sus necesidades pueden ser cubiertas por la leche materna, aunque se ha observado que la deficiencia en bebés prematuros puede ser la causa de anemia hemolítica (Gomez-Pomar et al., 2018). Algunos estudios han mostrado que una suplementación a dosis moderadas puede contribuir a reducir el desarrollo de la anemia hemolítica en estos lactantes (Brion et al., 2003). En otro estudio, el tratamiento con alfa tocoferol (25 UI / día) junto con suplementos de hierro (5 mg / kg / día) mejoró significativamente la anemia en recién nacidos prematuros (Arnon et al., 2009).

Elementos inorgánicos

El rápido crecimiento también se traduce en unos mayores requerimientos de minerales y elementos traza (Gibson & Hotz, 2000; Melø et al., 2008; Pandelova et al., 2012). La deficiencia de elementos inorgánicos durante la infancia produce signos y síntomas clínicos múltiples y diversos (Avila et al., 2016; Bertinato, 2016; Mazur & Maier, 2016).

Así, podemos observar por ejemplo que el calcio es esencial para una óptima mineralización ósea, pudiendo llegar a producirse raquitismo en niños con dietas deficientes en este mineral (FESNAD, 2010). Algunos estudios han mostrado que la leche materna aporta en torno a unos 200 - 300 mg de calcio por litro de leche al día (Rodríguez Rodríguez et al., 2002; Hicks et al., 2012; Olausson et al., 2012), cantidad que se considera adecuada para cubrir los requerimientos de dicho mineral durante los 6 primeros meses de vida de los lactantes. A partir de los 6 meses, los requerimientos de calcio se elevan hasta los 500 mg/día, por lo que la brecha de este requerimiento debe ser cubierta mediante la AC.

Es importante tener en cuenta ciertos factores dietéticos que pueden influir en la absorción de este elemento. Entre ellos, destacamos las proteínas, que según algunos estudios parecen tener un efecto negativo sobre su bioaccesibilidad (Anderson, 2004; Cámara et al., 2007). Este efecto se agravaría aún más en el caso de que la dieta fuera pobre en calcio. Por otro lado, es importante tener en cuenta que un exceso de calcio en la dieta puede influir en la absorción de otros minerales como hierro, zinc, manganeso y magnesio (FESNAD, 2010).

La deficiencia de hierro todavía se considera la deficiencia nutricional más común en el mundo, tanto en países desarrollados como en vías de desarrollo (Amaro López & Cámara Martos, 2004; Joshi et al., 2014). Durante los primeros años de la infancia, un bajo status de hierro se asocia a un menor desarrollo cognitivo, déficit en la atención y memoria, retraso psicomotor y problemas de comportamiento (Lönnerdal & Hernell, 2010). El tratamiento de este déficit de hierro no siempre es fácil y los problemas pueden persistir durante la edad escolar e incluso extenderse hasta la adolescencia (Grantham-McGregor & Baker-Henningham, 2010; Moráis López et al., 2011).

Durante la lactancia materna, el contenido de hierro presente en la leche materna es bajo, aunque su biodisponibilidad es alta, por lo que las necesidades del infante se ven cubiertas hasta los 6 meses (SEGHNP-AEP, 2010). A partir de los 6 meses, al igual que ocurre para otros nutrientes, la brecha que existe en las necesidades de hierro que ya no es cubierta con la leche materna, se complementará con la introducción de la AC. Por otro lado, debe destacarse que el hierro en la dieta humana se encuentra presente de dos formas; hierro hemínico (Fe-Hemo, que se une a la hemoglobina y mioglobina), presente en fuentes de alimentos animales como carne aves y mariscos; y hierro no hemínico (Fe-No Hemo, que puede estar presente en la forma de ferroso y férrico) presente en los alimentos de origen vegetal (McDermid & Lönnerdal, 2012) y también en alimentos de origen animal. El hierro hemo, se absorbe mejor que el hierro no hemo, pudiendo llegar a contribuir al 40% del total de hierro absorbido en la dieta (Hurrell & Egli, 2010). Por tanto, debe asegurarse una ingesta adecuada de alimentos de origen animal, que aporten hierro, especialmente del grupo hemo, de más fácil absorción (Moráis López et al., 2011).

También debemos recalcar la importancia tanto de la vitamina C como de las proteínas en la absorción del hierro no hemo. La mejora en dicha absorción por parte de la vitamina C, no solo es debido a la capacidad de reducir el hierro férrico a ferroso, sino que también se debe a su potencial para quelar el ion hierro manteniéndolo soluble en el lumen intestinal hasta el momento en que es absorbido (Teucher et al., 2004; Hurrell & Egli, 2010). Con respecto al efecto de las proteínas sobre la absorción de hierro, inicialmente llamado factor cárnico, en un principio se propuso que eran las proteínas de origen animal las implicadas en el aumento de la biodisponibilidad del hierro no hemo. El mecanismo mediante el cual las proteínas de la carne aumentaban la absorción de hierro no hemo se relacionaba con su contenido de aminoácidos azufrados, cisteína e histidina, ya que los grupos sulfidrilos promovían la solubilidad del hierro en estado ferroso e incluso facilitaban la reducción del hierro férrico a ferroso (Mulvihill & Morrissey, 1998; Baech et al., 2003). Las carnes, por su contenido en actina y miosina, tienen un alto contenido en estos aminoácidos azufrados y esta era la razón por la que el efecto promotor de las proteínas sobre la absorción de hierro se llamó en principio factor carne.

También debemos destacar que el consumo de algunas verduras como espinacas, acelgas, col, remolacha, debido a su contenido en nitratos, puede causar metahemoglobinemia. La capacidad de la metahemoglobina para transportar oxígeno es menor que la de la hemoglobina ferrosa (Fe^2), lo que se traduce en una menor llegada de oxígeno a los tejidos. Por ello, debemos tener la precaución de no introducir estos alimentos antes del año (SEGHNP-AEP, 2010; (Basulto et al., 2014). Además de ello, el alto contenido de fibra en estos alimentos de origen vegetal podría también afectar a la absorción de hierro si estos se introducen en la dieta del niño muy precozmente.

Por otro lado, el hierro ferroso es transportado a través de la membrana celular por la proteína transportadora de metales divalentes (DMT-1), uno de los transportadores solubles de membrana (*solute carrier*) conocido también como SLC11A2. La DMT-1 transporta también, de forma competitiva, otros iones divalentes como zinc, cadmio, plomo, manganeso, cobalto, níquel y cobre. Por lo tanto, un exceso de hierro en la dieta podría provocar un trastorno de la absorción o el metabolismo de otros elementos minerales (González García, 2013).

Otros minerales, como el zinc, muestran síndromes de deficiencia, tanto en niños (Luabeya et al., 2007), como en la población en general (Barnett et al., 2010). Las principales causas de deficiencia de zinc en niños se deben a una ingesta dietética insuficiente, nutrición parenteral a largo plazo sin suplementación y causas enterales como malabsorción (Willoughby & Bowen, 2014). Así, los bebés prematuros tienen un mayor riesgo de deficiencia debido a la insuficiente acumulación de reservas de zinc, así como un tracto gastrointestinal inmaduro, lo que resulta en un balance negativo de zinc con elevada excreción por el tracto intestinal (Finch, 2015).

Se ha observado que la deficiencia de zinc puede afectar al crecimiento, al sistema inmune y alterar la integridad y función del tracto gastrointestinal (Krebs et al., 2014). Otros síntomas que se pueden observar son dermatitis, diarrea, hipogonadismo, etc (Abrams, 2013); AAP, 2009). Debido a la similitud de los signos y síntomas de una carencia leve a moderada (mayor susceptibilidad a las infecciones y retraso del crecimiento) con otras carencias de nutrientes u otras enfermedades prevalentes de la infancia, puede ser difícil de diagnosticar (de Benoist et al., 2007; Brown et al., 2009).

Por otro lado, diferentes estudios han mostrado un efecto positivo de la suplementación de zinc y el crecimiento lineal en niños (Imdad & Bhutta, 2011; Hassan et al., 2012; Liu et al., 2018). Además, se ha observado que el zinc puede ser adecuado para tratar la diarrea en niños mayores de 6 meses, especialmente si tienen riesgo de deficiencia de zinc (Lazzerini, 2016).

Es importante destacar que existen ciertos factores que promueven o inhiben la biodisponibilidad de zinc. Así, entre los factores dietéticos que inhiben la absorción de zinc, encontramos algunos compuestos antinutricionales como el ácido fítico (Krebs et al., 2014; Pechin, 2012). El efecto inhibidor del ácido fítico, se debe a la formación de quelatos entre los grupos fosfato y los iones zinc (Cámara Martos et al., 2015). Entre los factores promotores de la bioaccesibilidad del zinc, se encuentran las proteínas (Jovani et al., 2000; Pérez-Llamas et al., 2003). Al igual que ocurría para el hierro, los productos de digestión de las proteínas son capaces de formar compuestos solubles con el zinc, facilitando su absorción por los enterocitos. También la presencia de aminoácidos azufrados como cisteína e histidina promueven la absorción de este elemento (Cámara Martos et al., 2015).

Al igual que los anteriores micronutrientes, otro elemento traza que debemos destacar durante la infancia es el cobre. Tiene un papel muy importante ya que actúa como cofactor de muchas enzimas, tales como ceruloplasmina, elastasa, citocromo oxidasa y superóxido dismutasa (Domellöf et al., 2018). Síndromes de deficiencia de cobre se pueden detectar en los lactantes debido a una pobre ingesta de este elemento en la dieta (Lönnerdal, 2005; Olivares et al., 2010). Entre los síntomas podemos destacar que su deficiencia ha sido asociada a anemia, neutropenia y cambios óseos en lactantes desnutridos (Müller & Tanner, 2017). En mayor medida, es probable que la deficiencia de cobre ocurra en niños prematuros, los cuales tienen un mayor requerimiento de cobre debido al rápido crecimiento y a que sus reservas hepáticas se ven reducidas (de Romaña et al., 2011) (este elemento traza se acumula en el hígado principalmente durante el tercer trimestre, (Gil Hernández et al., 2006). Otras enfermedades relacionadas con la alteración del cobre, tanto por defecto como por exceso, han sido detalladas por otros autores (Araya et al., 2003). Así, encontramos la Enfermedad de Menkes, descrita en la década de los sesenta por John Menkes. Su prevalencia se estima en 1/100.000-250.000 recién nacidos (Ros & Ros, 2010; Feoktistova Victorava & Clark Feoktistova, 2018). Esta enfermedad se debe a una mutación en el gen que codifica el transportador *ATP7A*, que impide la salida de cobre a las células intestinales (Ros & Ros, 2010; Müller & Tanner, 2017). Algunos de los síntomas en neonatos son la ictericia, hipotermia, hipoglucemia y el deterioro neurológico (Feoktistova Victorava & Clark Feoktistova, 2018). También ha sido descrita su toxicidad, siendo su principal síntoma el daño en el hígado (de Romaña et al., 2011; Gaetke et al., 2014). Así, Samuel Alexander Wilson en 1912 describió la Enfermedad de Wilson. Esta enfermedad cuya prevalencia se estima en 1/30000 recién nacidos, es causada por una mutación del gen que codifica el transportador ATP7B y principalmente provoca una acumulación de cobre en hígado y cerebro (Ros & Ros, 2010).

Otro aspecto a considerar es la interacción cobre – hierro, ya que se ha demostrado que la homeostasis del cobre está estrechamente relacionada con el metabolismo del hierro, debido a que ambos poseen propiedades fisicoquímicas y toxicológicas similares (Collins et al., 2010; Ha et al., 2016). Un estudio realizado con líneas celulares Caco-2 ha sugerido que el cobre disminuye la biodisponibilidad de hierro no hemo como consecuencia de que ambos metales utilizan para su absorción el mismo transportador de membrana apical (DMT1) (Arredondo et al., 2006). Por otro lado, se sabe que diferentes enzimas dependientes de cobre (como por ejemplo la ceruloplasmina) están implicadas en la absorción intestinal y la movilización del hierro entre los distintos tejidos (Sharp, 2004). De esta forma, la deficiencia en cobre afectaría a la biodisponibilidad de hierro.

Respecto al manganeso, también es un elemento traza a destacar puesto que actúa como cofactor de numerosas enzimas implicadas en el crecimiento y desarrollo óptimo (Finch, 2015; Andiarena et al., 2020). Las principales fuentes de este elemento en la dieta son alimentos vegetales como legumbres, cereales y frutas secas (ATSDR, 2012). Existen pocos estudios sobre la deficiencia de manganeso en humanos. Sin embargo, la toxicidad del manganeso ha empezado sí ha sido ampliamente estudiada en la población infantil (Roels et al., 2012; Grandjean & Landrigan, 2014; Lucchini et al., 2017). Se ha planteado que altas exposiciones de manganeso podrían tener efectos adversos sobre el desarrollo neurológico infantil (Frisbie et al., 2019; Andiarena et al., 2020). Esta toxicidad puede ser más acusada en bebes (especialmente neonatos) debido a la reducción transitoria de la excreción biliar, ruta principal de excreción del manganeso en humanos (Neal & Guilarte, 2013). Sin embargo, esta asociación entre altos niveles de manganeso y ciertos efectos de neurodesarrollo, no es completamente concluyente (Leonhard et al., 2019).

Debemos destacar las posibles interacciones que se producen entre este elemento y otros micronutrientes como hierro y zinc. Aunque los mecanismos para la absorción y transporte del manganeso no están claros, sí que existe cierta evidencia de que el hierro puede compartir vías de absorción y transportes comunes (Fitsanakis et al., 2010). Así, estudios previos han mostrado una influencia negativa de un alto contenido de hierro presente en la dieta sobre la biodisponibilidad de manganeso (Aschner & Aschner, 2005; Intawongse & Dean, 2006).

La presencia de ácido fítico en alimentos de origen vegetal también puede disminuir la bioaccesibilidad de manganeso (Lönnerdal, 2002). No obstante, algunos estudios realizados con alimentos listos para consumir (Agte et al., 2005; Velasco-Ryenold et al., 2008) han indicado que los tratamientos de procesado y cocinado disminuyen este efecto inhibitorio sobre el manganeso al producirse la hidrólisis del ácido fítico en compuestos tri y difosforilados.

Con relación al magnesio, se encuentra ampliamente distribuido en la naturaleza. Así, son ricos en magnesio la mayoría de vegetales verdes, frutos secos como las nueces, legumbres frescas, oleaginosas y granos integrales (Pérez González et al., 2009). Los valores de referencia están bien definidos para los adultos. No ocurre lo mismo para el caso de los recién nacidos y prematuros, cuyos valores son muy limitados (Rigo et al., 2017). Aunque su deficiencia es rara, puede darse en niños con trastornos endocrinos como la diabetes mellitus o con desordenes gastrointestinales como los síndromes de malabsorción (Serefko et al., 2016), así como niveles insuficientes obtenidos a través de la dieta. Entre los síntomas que pueden ser asociados a la falta de magnesio en los niños encontramos provoca alteraciones cardiovasculares, gastrointestinales, renales, musculares, neurológicas, inmunes.

Por otro lado, la toxicidad de magnesio (hipermagnesemia) en niños suele ser rara. Dicho exceso ha sido observado en algunos recién nacidos, debido a que el sulfato de magnesio es utilizado en embarazadas para el tratamiento de la preeclampsia materna o parto prematuro (Cruz et al., 2009; Doyle et al., 2009; Menéndez-Hernando et al., 2019). La mayoría de neonatos con hipermagnesemia son asintomáticos, aunque algunos de los síntomas que se observan son neurológicos como hipotonía, disminución de la succión, reflejos atenuados, llanto débil o neuromuscular con debilidad y letargia, también cardiovasculares y apnea entre otros (Menéndez-Hernando et al., 2019). Aunque la hipermagnesemia ha sido asociada a parálisis intestinal en adultos, en los recién nacidos no se ha logrado establecer este efecto con total seguridad (Cruz et al., 2009).

El metabolismo del magnesio está ampliamente relacionado con el calcio y el potasio (Serefko et al., 2016). La deficiencia severa de magnesio puede ser la causa de hipocalcemia e hipopotasemia (Tong & Rude, 2005; Alcázar Arroyo et al., 2011). Por tanto, en su bioaccesibilidad debe ser considerada la cantidad de ambos minerales. Además, como sucede para algunos de los elementos descritos anteriormente, se ha observado que existen varios factores que pueden favorecer su bioaccesibilidad. Tal es el caso de la vitamina C, como consecuencia de la formación de complejos solubles entre el ácido ascórbico y el magnesio (Velasco-Reynold et al., 2010).

El selenio es otro de los minerales que debemos tener en cuenta durante este período. Una malabsorción o el uso de dietas especiales con un contenido insuficiente de selenio pueden provocar síntomas como un deterioro en la función muscular o pérdida del pigmento en el cabello y la piel. En bebés y niños pequeños, un bajo estado de selenio se ha relacionado con un retraso en el desarrollo cognitivo (Skröder et al., 2015; Polanska et al., 2016). Sin embargo, puede llegar a ser tóxico a niveles elevados. Un exceso de selenio se caracteriza por pérdida del cabello, dermatitis y síntomas endocrinológicos y neurológicos entre otros (EFSA, 2013).

La bioaccesibilidad del selenio puede verse comprometida por factores dietéticos como la grasa (Marval-León et al., 2014). Este efecto inhibidor, se debe a que las especies de selenio son moléculas pobremente lipofílicas (Moreda-Piñeiro et al., 2013), y las micelas grasas que se forman durante el proceso de digestión pueden interferir con la capacidad de las enzimas para liberar selenio unido a péptidos de bajo peso molecular (Marval-León et al., 2014).

Por otro lado, cabe destacar el posible efecto beneficioso del selenio sobre diferentes metales pesados como por ejemplo el cadmio. Esta interacción, ha sido mostrada en estudios previos (Pappas et al., 2011; Marval-León et al., 2014), y se debe a la formación de complejos o sales selenio – metal pesado insolubles o poco solubles en agua. También dependerá de las concentraciones iniciales en que se encuentren ambos elementos (ratio Se/Cd) y la forma química en la que se encuentra presente el selenio (Cámara-Martos et al., 2019).

Por todo lo anterior la AC juega un papel muy importante en el suministro adecuado de micronutrientes inorgánicos. A partir de los 9 meses, por ejemplo, la AC debe proveer entre el 75-100% de los requerimientos de hierro y zinc (Melø et al., 2008). Según la EFSA (2013), entre los 6 y los 12 meses se recomiendan aportar de 6 a 11 mg/día de hierro. Para el tratamiento de niños con diarrea se recomienda una suplementación de zinc (durante un periodo de 10 – 14 días) de 10mg/día en el caso de lactantes menores de 6 meses y de 20 mg/día para mayores de 6 meses (WHO/UNICEF, 2004).

1.2 ALIMENTACIÓN ECOLÓGICA

Actitud de los consumidores frente a la alimentación ecológica

Durante los últimos años, la población mundial ha experimentado un crecimiento exponencial. Es por ello que uno de los debates más discutidos en la actualidad es el de cómo alimentar a dicha población, no solo a las generaciones presentes sino también a las futuras. Además, preocupa que la consecución de dicho objetivo no sea a expensas de la degradación del medio ambiente. Por todo ello y debido a la creciente preocupación social con la sostenibilidad, numerosos estudios (Goldberger, 2011; Halberg, 2012; Lockie, 2006; Niggli, 2014) han sido llevados a cabo con el fin de elucidar si la agricultura ecológica podría ser de utilidad.

Sin embargo, se han obtenido resultados contradictorios. Así, diferentes autores han mostrado unos rendimientos inferiores en cultivos de arroz, soja, maíz y trébol ecológico (6 – 11%) y en frutas y trigo (28% y 27%) con respecto a la agricultura convencional (de Ponti et al., 2012). Otro estudió también mostró rendimientos más bajos para frutas y semillas ecológicas (3% y 11% respectivamente) y para los cereales, vegetales y trigo ecológicos (26%, 33% y 37% respectivamente) cuando se les compara con los cultivos convencionales (Seufert et al., 2012).

En contraposición, otros autores han mostrado que la agricultura ecológica no solo logra el mismo rendimiento que la convencional para algunas variedades de tomates (Liebhardt, 2001) manzanas (Reganold et al., 2001) y vegetales de hoja (Brassica rapa L. cv. Kairyo y Brassica campestris L. cv.) (Xu et al., 2003), sino que a veces este rendimiento es incluso mayor para cereales como el trigo (45%) (Leu, 2004) maíz (30%), soja (65%) y frutas como el mango (75%) o vegetales como el repollo (21%) (Pretty & Hine, 2001).

A día de hoy, dado que los consumidores son más conscientes de los peligros para la salud, de la presencia de pesticidas y otros contaminantes químicos en los productos agrícolas, no es de extrañar que estos productos ecológicos se estén empezando a considerar como más saludables por un sector de población cada vez mayor. Este cambio de dirección hacia el consumo de productos ecológicos se transmite en especial al entorno familiar y por ende influye también en la alimentación infantil (Hill & Lynchehaun, 2002).

¿Qué son los alimentos ecológicos?

Los alimentos ecológicos, también conocidos como orgánicos por su traducción al inglés, pueden definirse como aquellos productos alimenticios, obtenidos en explotaciones agrarias y/o ganaderas en los que para su obtención no se ha empleado ningún tipo de producto químico sintético, hormonas, agentes antibióticos, modificaciones genéticas o irradiación (de Souza Araújo et al., 2014; Galiano Segovia & Moreno Villares, 2016). La FAO define la producción de alimentos ecológicos como «un sistema holístico de manejo de la producción que promueve y facilita la salud agroecológica, incluyendo la biodiversidad, los ciclos biológicos y la actividad biológica del suelo» (Codex Alimentarius, 2007).

Por otro lado, debe destacarse que la agricultura ecológica debe seguir una regulación bien definida y el modo de producción debe ser controlado por un organismo de certificación (Codex Alimentarius, 2007; EC, 2016; USDA, 2016; IFOAM, 2017).

A continuación, en la siguiente figura (Figura 3) se muestran diferentes características que definen lo que es la producción de alimentos ecológicos.

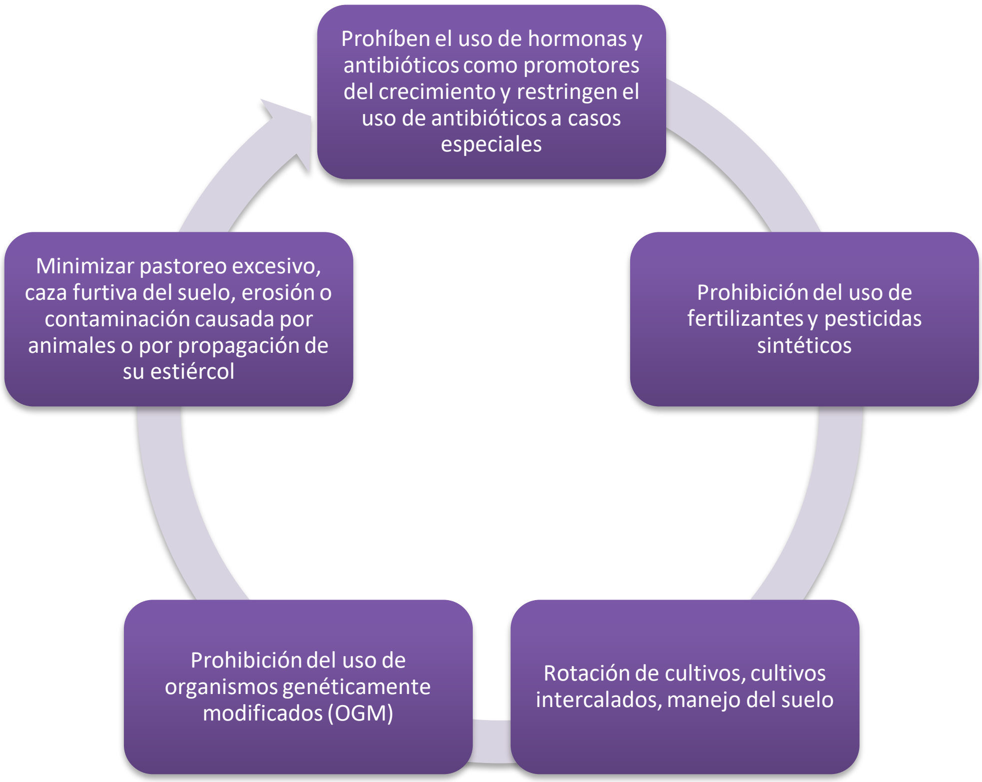


Figura 3: Características de la producción ecológica

Sus principales limitaciones son el uso mínimo de insumos no agrícolas, la prohibición de productos químicos sintéticos (fertilizantes, pesticidas, insecticidas, herbicidas, fungicidas, drogas), el uso de fertilizantes orgánicos y pesticidas naturales, largas rotaciones de cultivos, el mantenimiento de la materia orgánica y la vida microbiana en el suelo y la prohibición de plantas genéticamente modificadas (Guéguen & Pascal, 2016; Gomiero, 2018; Sharma & Singhvi, 2018).

Para la cría de animales, las regulaciones ecológicas se refieren al uso de alimentos ecológicos y la limitación de los tratamientos terapéuticos (especialmente antibióticos y hormonas). Además, pone gran atención en el bienestar animal. Así, la densidad de población está estrictamente regulada, y los animales deben tener acceso al aire libre o áreas de pastoreo siempre que sea posible (Gomiero, 2018). Ha sido reportado que el ganado alimentado con pastos tiende a ser menos vulnerable a las infecciones que ganado alimentado con granos (Pimentel, 2010).

Con respecto a los antibióticos, en la ganadería convencional, son empleados para prevenir infecciones en el ganado, ya que éstas pueden propagarse fácilmente debido a la alta densidad de población de animales bajo un régimen de producción intensivo (vacas lecheras, gallinas ponedoras). En algunos países, los antibióticos también se utilizan como promotores del crecimiento. El uso de hormonas y antibióticos como promotoras del crecimiento, está prohibido por las regulaciones ecológicas, restringiendo severamente el uso de antibióticos a casos especiales (Galiano Segovia & Moreno Villares, 2016). Por tanto, aquellos animales tratados mediante antibióticos, no se podrán comercializar como productos ecológicos certificados, debiéndose respetar un tiempo de espera adecuado (Gomiero, 2018).

Sin embargo, cabe señalar que los estándares pueden variar entre regiones. Los estándares de la UE difieren ligeramente de los estándares de EE. UU. Así, el uso de hormonas de crecimiento como la somatotropina sintética, está permitida en los EE. UU., mientras que está prohibido en otros países (Canadá y la Unión Europea) (Guéguen & Pascal, 2016).

Otra de las diferencias en los estándares, la encontramos en el etiquetado. En el etiquetado norteamericano se distinguen varias categorías de productos: 100% ecológico; ecológico (al menos el 95% de los ingredientes son procesados orgánicamente), y hecho con ingredientes ecológicos (al menos el 75%). Sin embargo, En Europa, sólo se considera producto ecológico cuando más del 95% de su composición es orgánica, y lleva el logotipo europeo de «producción orgánica» *(organic farming)* (Galiano Segovia & Moreno Villares, 2016).

A la hora de adquirir estos productos ecológicos, uno de los principales inconvenientes que nos encontramos es su elevado precio, el cual puede llegar a suponer una media de un 40% superior al de los obtenidos por sistemas de producción convencionales (Galiano Segovia & Moreno Villares, 2016). Las razones principales para este mayor precio son que dado que los costos de producción son más altos, los precios de venta también deberán ser significativamente más elevados (Guéguen & Pascal, 2016).

A pesar de esto, gran parte de la población ha demostrado estar dispuesta a pagar estos precios más altos por los productos ecológicos (Zander & Hamm, 2010). Se ha informado que los consumidores compran alimentos ecológicos porque creen que son más saludables (Hughner et al., 2007) y poseen una mayor calidad del producto (Kahl et al., 2012). Además, la demanda de dichos productos también aumenta constantemente ya que los consumidores los perciben como más respetuosos y seguros para el medioambiente (Aertsens et al., 2009; Honkanen et al., 2006; Læssøe et al., 2014; Ruiz de Maya et al., 2011).

Finalmente, en los últimos años se ha podido comprobar que la agricultura ecológica es uno de los sectores que más rápido ha crecido (Popa et al., 2019; Rembiakowska, 2016), hecho que se justifica con el crecimiento del número de productores y la superficie dedicada a estos cultivos en todo el mundo (Sharma & Singhvi, 2018). A nivel mundial, la agricultura ecológica en 2014 se practicaba en 172 países, suponiendo un total de 44 millones de hectáreas (1% de la tierra cultivada) según (IFOAM, 2016). Según los datos de Eurostat, en 2014 la Unión Europea tenía una superficie total de 10,3 millones de hectáreas cultivadas, frente a los 5,7 millones en 2002 (EUROSTAT, 2016). En algunos países europeos (Dinamarca, Suiza y Austria) los alimentos ecológicos ahora representan un porcentaje bastante significativo (alrededor del 7%) del mercado (EC, 2014; IFOAM, 2016).

Valor nutricional de los productos ecológicos

A día de hoy, como hemos dicho anteriormente, existe la convicción popular de que los alimentos ecológicos poseen un mayor valor nutricional. Fuera de las motivaciones de elección de los consumidores, éticas o ideológicas, es importante saber si dicha creencia generalizada de que dichos alimentos tienen una mejor calidad nutricional está justificada desde el punto de vista científico. En este contexto, señalaremos diferentes variables que pueden afectar la calidad del producto final.

Humedad

La materia seca es un indicador importante para medir la acumulación de materia orgánica y la composición nutricional, incluyendo almidón, celulosa, proteínas, grasas, minerales y otros elementos inorgánicos, etc. Diferentes estudios han mostrado como las frutas y verduras ecológicas contienen un mayor contenido en materia seca que las convencionales (Huber et al., 2011; Lairon, 2010). Este hecho, puede deberse a que las plantas convencionales crecen consumiendo fertilizantes químicos excesivos, para lo cual necesitan absorber más cantidad de agua (Herencia et al., 2011).

Así, Lombardo et al., (2012), mostraron como el contenido en materia seca de patatas ecológicas era superior (22,2%) al de las patatas convencionales (20,3%). De igual modo, Pieper & Barrett, (2009), observaron como en tomates ecológicos el contenido en materia seca era entre un 4 – 20 % mayor que el de tomates convencionales). También, Gąstoł et al., (2011), señaló que el contenido de materia seca es mayor en frutas y verduras ecológicas como grosellas negras, peras, remolachas y apio (15,2%, 12,0%, 12,2%, y 10,4% respectivamente frente al 12,6%, 11,2%, 8,3% y 8,9% de las convencionales), pero menor en zanahorias y manzanas ecológicas en comparación con productos convencionales.

Sin embargo, también hay estudios que demuestran que los productos ecológicos poseen un contenido en materia seca menor que los productos convencionales. Por ejemplo, Brazinskiene et al., (2014) observaron que patatas convencionales pueden contener mucha más materia seca (entre un 1,3 – 28,5% más) que las ecológicas.

Proteínas

Generalmente, el contenido de proteínas suele ser más bajo en los productos ecológicos en comparación al contenido encontrado en los productos convencionales. Esto es debido principalmente a la insuficiencia en el suministro de fertilizantes nitrogenados (Yu et al., 2018). La mayoría de los estudios realizados en cereales encuentran unos niveles más pobres en proteínas en cultivos ecológicos. Tal fue el caso del estudio realizado por Vrcek et al., 2014, en el cuál encontraron un contenido en proteína más bajo (14%) en harinas de trigo ecológicas en relación a las convencionales.

Sin embargo, esta tendencia no es sistemática puesto que Carillo et al., (2012) han encontrado no solo un contenido de proteínas mayor en patatas ecológicas frente a las convencionales (32,9%), sino que la calidad de estas proteínas también fue superior con un mayor contenido en aminoácidos esenciales (20,4%).

Lípidos

Uno de los factores que puede afectar al contenido de ácidos grasos de la carne es la alimentación (Guéguen & Pascal, 2016). Así, la composición de ácidos grasos de los piensos influye en la composición de ácidos grasos de huevos, leche y especialmente en el contenido de ácidos grasos insaturados de la carne; mucho más en cerdos y aves de corral que en rumiantes (Popa et al., 2019). Por tanto, la carne del ganado alimentado principalmente con pastos o forraje fresco (preferido en la cría ecológica), es más rica en ácidos grasos poliinsaturados, principalmente ω-3 que la carne del ganado alimentado con ensilaje o concentrado de maíz (Guéguen & Pascal, 2016). Angood et al., (2008) mostraron que chuletas de cordero ecológicas tienen un mayor contenido de ácidos grasos totales (3430 mg/100 g) y poliinsaturados (133 mg/100 g para ω3) que chuletas de cordero convencionales: totales (2960 mg/100 g); poliinsaturados (111 mg/100 g). De igual modo, en carne de res bovina ecológica (novillos lecheros), se ha observado un menor porcentaje de triglicéridos y ácidos grasos saturados (77,9% y 27,2%) en comparación con la carne de res no ecológica (86,6% y 35,2%) y al mismo tiempo un mayor porcentaje de ácido oleico (47,1%), ácido α-linolénico (0,2%) y ácidos grasos w-3 (0,2%) frente a 39,9%, 0,1 y 0,2% del contenido de ácido oleico, α-linolénico y w-3 respectivamente de la carne convencional (Bjorklund et al., 2014).

Vitaminas

En relación a las vitaminas, nutrientes esenciales en el mantenimiento del cuerpo humano para llevar a cabo diferentes funciones fisiológicas, Vinha et al., (2014) concluyó que la cantidad de Vitamina C en tomates ecológicos era un 30% superior a la de tomates producidos de manera convencional. De igual modo, Oliveira et al., (2013) han mostrado mayores niveles de esta vitamina (55%) en tomates ecológicos.

Sin embargo, esta tendencia no es sistemática y otros autores no han encontrado diferencias significativas en el contenido de vitamina C de tomates (Hallmann et al., 2013) o fresas (Crecente-Campo et al., 2012) producidas tanto de manera convencional como de manera ecológica. En contraposición a todo lo anterior, otros estudios han mostrado un contenido más alto de vitamina C en patatas (Lombardo et al., 2012) y zanahorias convencionales (Bender et al., 2009) cuando se las compara con las ecológicas (24%).

Compuestos antioxidantes

Este tipo de compuestos, entre los cuales destacan los compuestos fenólicos o polifenoles, son un grupo de fitoquímicos ampliamente difundidos en el reino vegetal y tienen una importancia fisiológica y morfológica relevante para las plantas (Popa et al., 2019). Además, son de considerable interés para la dieta humana debido a su relación con un menor riesgo de enfermedades crónicas neurodegenerativas y ciertos tipos de cáncer (Barański et al., 2014; Talhaoui et al., 2015; Popa et al., 2019) así como en el desarrollo de alimentos funcionales. Dentro de estos compuestos antioxidantes se incluyen los flavonoides (antocianinas, flavonol, flavonoides, etc.), terpenoides (carotenoides, luteína) y compuestos nitrogenados (glucósido, aminas, alcaloides, etc.) (Li et al., 2012).

Se ha sugerido que las diferencias en el contenido de compuestos antioxidantes en vegetales podrían deberse al impacto de diferentes prácticas de fertilización en el metabolismo de las plantas y al hecho de que las plantas cultivadas bajo prácticas ecológicas tienen que sintetizar sus propias defensas químicas (Winter & Davis, 2006; Barański et al., 2014). Es decir, en ausencia de tratamiento fitofarmacéutico, el aumento de los compuestos antioxidantes del cultivo ecológico puede explicarse por una reacción de defensa de la planta, sin ninguna protección, contra ataques de insectos u hongos (Guéguen & Pascal, 2016). Además, se ha observado que el empleo de fertilizantes ecológicos produce diferencias significativas en los patrones de expresión de genes y proteínas y, como resultado, en perfiles de metabolitos secundarios (Tétard-Jones et al., 2013; Barański et al., 2014). Del mismo modo, la baja disponibilidad de nitrógeno en el suelo también podría tener el mismo efecto, promoviéndose, por tanto, la producción de metabolitos secundarios, incluidas moléculas con acción antioxidante beneficiosa (Guéguen & Pascal, 2016).

En consecuencia, los niveles de polifenoles, especialmente flavonoides en frutas y verduras ecológicas son a veces entre 20 y 40% mayores en los vegetales cultivados de forma ecológica (Mitchell et al., 2007; Vallverdú-Queralt et al., 2012; Hallmann et al., 2013).

Elementos inorgánicos

Diferentes cultivos de una misma cosecha pueden diferir en su composición en micronutrientes, dependiendo de la variedad utilizada, el régimen de pesticidas y fertilizantes empleado, las condiciones de crecimiento, la estación y otros factores (Johansson et al., 2014). Además, cabe destacar que las prácticas agronómicas utilizadas en los sistemas de agricultura ecológica pueden afectar los patrones de absorción de minerales y los procesos metabólicos de las plantas cultivadas (Gomiero, 2018).

Así, un estudio reveló que variedades de tomates ecológicos tenían unas mayores concentraciones de K (4,5%), Ca (129,8%), Zn (65,4%) que las convencionales (Sheng et al., 2009). De igual manera, de Souza Araújo et al, 2014, observaron en pimientos verdes y lechuga ecológicos un mayor contenido de K (28%, 25%), Mg (20%, 19%), Na (45%, 13%) y Cr (67%, 60%). Otro estudio realizado con harina de trigo ecológica mostró que contenía más K (11,1 – 8,1%), Mg (14,5 – 9,1%), Zn (12,4 – 15,0%), Ni (16,3 – 9,2%), Mo (31,6 – 41,1%) en comparación con las convencionales para los años 2008 y 2009 respectivamente (Vrček et al., 2014).

Pesticidas, metales pesados y nitratos

Por otro lado, debe considerarse que los productos obtenidos mediante las técnicas de agricultura convencional emplean pesticidas. Los pesticidas son sustancias que se usan para destruir u obstaculizar la acción de una plaga o para ejercer un control efectivo de los organismos objetivo (de Souza Araújo et al., 2014). Principalmente se incluyen organoclorados y organofosforados (Wu et al., 2011; Duan & Shao, 2014). Muchos de esos compuestos se degradan lentamente en el medio ambiente (en muchas ocasiones durante largos años) y, por lo tanto, se acumulan en el suelo, agua y otros organismos vivos (AAVV, 2013; Fenner et al., 2013). Es importante destacar que, debido a su persistencia en el medio ambiente, los residuos de algunos pesticidas prohibidos hace mucho tiempo todavía se encuentran en los alimentos (EFSA, 2016). Son bien conocidos los efectos negativos (cancerígenos, tóxicos para el sistema reproductivo, disrupción endocrina) que pueden tener dichos pesticidas sobre la salud, (PAN, 2009; Mie et al., 2017), afectando a la mayoría de órganos y tejidos tanto como exposición aguda como por exposición crónica; y no solo en los agricultores que manejan estos productos sino también en los consumidores de estos alimentos, dando lugar a la aparición de enfermedades graves tales como cáncer, enfermedad de Parkinson (van Maele-Fabry et al., 2012) resistencia a la insulina y obesidad (Lim et al., 2009). Por ello, el control riguroso de los efectos adversos de los pesticidas en los humanos y el medio ambiente es fundamental. Para controlar el uso de pesticidas, se establecieron estándares y límites de tolerancia máxima en diferentes países (de Souza Araújo et al., 2014).

Además, cabe señalar en el sistema agrícola convencional, la mayoría de los fertilizantes químicos, pesticidas y forrajes contienen metales pesados, por lo que el empleo de pesticidas en la agricultura convencional, provoca el aumento del contenido de metales pesados en el suelo, absorbiéndose por los cultivos y concentrándose en sus partes comestibles. Los metales pesados, después de ingresar a través de la cadena alimentaria se acumulan en el cuerpo humano y pueden llegar a causar un deterioro crónico sin ser detectados (Yu et al., 2018). Debido a que la agricultura ecológica prohíbe estrictamente la aplicación de materiales nocivos para el ecosistema agrícola, el contenido de metales pesados es relativamente más bajo en los productos ecológicos (Yu et al., 2018).

Del mismo modo, se debe considerar que la presencia de estos metales pesados en productos convencionales utilizados en la alimentación complementaria, pueden plantear riesgos para la salud de los niños (Tripathi et al., 2001; Souad et al., 2006; Roberts et al., 2012). Los bebés tienden a estar expuestos a niveles relativamente más nocivos de productos químicos de alimentos, ya que, en relación con su peso corporal, consumen más alimentos que los adultos. Algunos de estos contaminantes pueden producir disrupciones hormonales, llegando a provocar modificaciones en determinados genes importantes para su desarrollo(Howard, 2003; Mnif et al., 2011).

Entre los metales pesados más tóxicos nos encontramos al cadmio y plomo, elementos que, como consecuencia del uso de los pesticidas, se encuentran muy expandidos en la naturaleza. Se ha demostrado que, a través de la leche materna, la exposición al cadmio podría inducir en la vida temprana del lactante a estrés oxidativo y asociarse a una menor inteligencia infantil (Kippler et al., 2012). Además, el cadmio podría almacenarse en diferentes órganos (pulmones, riñón, sistema digestivo, tejido óseo, gónadas) y permanecer en el cuerpo de un niño hasta la edad adulta (Sughis et al., 2011). También se ha identificado como un potente neurotóxico (Rodríguez-Barranco et al., 2013).

Algunos autores han reportado la presencia de unos niveles más bajos de cadmio en productos ecológicos cuando se les compara con los productos convencionales. Así, un estudio realizado por Vrček et al., 2014 mostró que en harina de trigo ecológica en los años 2008 y 2009 existía un menor contenido de cadmio (38,2 y 30,7%) y plomo (34,7 y 48,0%), que en la harina de trigo convencional. Del mismo modo, de Souza Araújo et al., (2014) mostró un contenido de plomo menor para lechugas (15,7%) y tomates ecológicos (2,3%) frente a convencionales. Esto puede ser debido a que muchos de los fertilizantes empleados en la agricultura convencional están contaminados con dicho metal(Barański et al., 2014; McCarty & DiNicolantonio, 2014).

A medida que el cadmio se perfila como una causa importante de trastornos vasculares, como casos de infartos de miocardio, enfermedades cerebrovasculares (Everett & Frithsen, 2008), casos de enfermedad coronaria, accidentes cerebrovasculares e insuficiencia cardiaca (Agarwal et al., 2011; Tellez-Plaza et al., 2013), varios tipos de cáncer comunes (Gallagher et al., 2010; Hartwig, 2013; Nagata et al., 2013; Strumylaite et al., 2014), enfermedades renales y daño hepático (Järup & Åkesson, 2009), estrés oxidativo (Cuypers et al., 2010) y osteoporosis (Nawrot et al., 2010; McCarty, 2012), el hecho de que los productos ecológicos tengan unos niveles más bajos de cadmio es un hecho relevante para la salud (McCarty & DiNicolantonio, 2014).

Sin embargo, también es necesario señalar que en otro estudio no se encontraron diferencias significativas con respecto al contenido de metales pesados como cadmio, plomo, arsénico o mercurio cuando se compararon productos ecológicos y convencionales en vegetales como lechuga, zanahorias, patatas y productos derivados del cerdo, carne de vaca y de gallina (Hoogenboom et al., 2008). Debe especificarse que el contenido de cadmio en los cultivos también depende de su concentración nativa en el suelo.

Por otro lado, también debemos destacar la presencia de nitratos como compuestos no deseables. La mala reputación de los nitratos se debe a accidentes pasados de metahemoglobinemia en bebés con mala higiene de los alimentos, especialmente biberones contaminados, en los que los microorganismos aceleran la reducción de nitrato a nitrito (Guéguen & Pascal, 2016).

El nitrato es abundante en vegetales. Así, la ingesta del 80% de nitrato de la dieta diaria procede de las verduras (Yu et al., 2018). Varios estudios han demostrado como el contenido en nitratos en los alimentos vegetales convencionales es mayor cuando se les compara con los ecológicos. Por ejemplo, Campbell & López-Ortíz, (2014), mostró que el contenido de nitratos de cuatro tipos de vegetales (col, zanahoria, perejil y col roja) era 3-5 veces mayor en los convencionales frente a los ecológicos. También, se descubrió que el contenido de nitrato de patatas ecológicas era un 35% menor que el presente en convencionales (Lombardo et al., 2012). También, Barański et al. (2014) ha indicado que las concentraciones de nitrógeno total y compuestos tóxicos como nitratos fueron más bajos en cereales ecológicos en relación con los cereales convencionales.

Sin embargo, otros informes también han mostrado respecto a su contenido en nitratos que no existían diferencias entre los productos ecológicos y convencionales (Yu et al., 2018). Esta circunstancia puede ser debida al hecho de que los fertilizantes ecológicos empleados en la agricultura ecológica, son rápidamente asimilados por la planta y, por tanto, también conducen a altos niveles de nitratos (Guéguen & Pascal, 2016).

Micotoxinas

Las micotoxinas son metabolitos secundarios tóxicos producidos por ciertos mohos bajo algunas condiciones ambientales, que pueden colonizar los cultivos tanto en el campo como durante su almacenamiento (Gomiero, 2018; Yu et al., 2018). Estos metabolitos secundarios tienen una fuerte toxicidad y algunos efectos cancerígenos, teratogénicos y mutagénicos, incluyendo principalmente la toxina de *Aspergillus flavus*, Aflatoxinas B1 y B2 (AF), el ketene giberélico de maíz (zearalenona, ZEN), solución de desoxinivalenol (DON), toxina HT-2 y toxina T-2 (Yu et al., 2018).

Sería lógico pensar que los cultivos ecológicos son más susceptibles a una presencia elevada de micotoxinas, dado que, en la agricultura ecológica los fungicidas sintéticos no están permitidos. Sin embargo, determinados estudios indican que no hay evidencias para apoyar dicha afirmación (FAO, 2000; Hoogenboom et al., 2008; Smith-Spangler et al., 2012). Así, un estudio realizado por Gourama, (2015), en el cual se analizaron 50 muestras ecológicas y 50 convencionales de diferentes productos (palomitas, arroz, maíz, nueces, almendras, cacahuetes, semillas de calabaza, guisantes, semillas de lino, soja y anacardos) reflejó que no existían diferencias entre ambos sistemas. Igualmente, en los Países Bajos, un estudio comparativo entre productos ecológicos y convencionales (trigo, lechuga, zanahorias, papas, cerdos, vacas y gallinas) no ha mostrado diferencias entre ambos tipos de cultivos con respecto a su contenido en micotoxinas (Hoogenboom et al., 2008).

1.3. LEGUMINOSAS

Introducción

La familia de las leguminosas está formada por casi 20.000 especies (Lewis et al., 2013; Llamas & Acedo, 2016) aunque solo algunas de ellas son consumidas por los humanos. Son un alimento esencial, que ha sido empleado desde la más remota antigüedad y aunque en un principio fueron empleadas como forraje para los animales, a día de hoy constituyen la base de la dieta de numerosos países (Sarmento, 2012). Pertenecen a la familia Fabaceae, conocidas en un principio como Leguminosae. Esta, a su vez se divide en tres subfamilias (Papilionoideae, Mimosoideae, Caesalpinioideae), siendo la Papilionoideae la más amplia y prácticamente la única cuyas especies se cultivan para el consumo humano (Figura 4) (López Amorós, 2000; van Wyk & Boatwright, 2013; Llamas & Acedo, 2016).

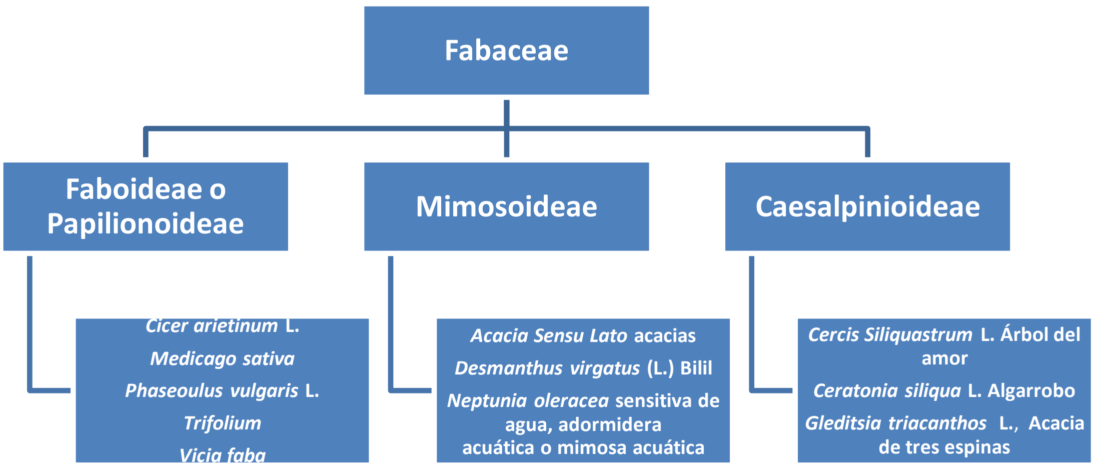


Figura 4. Clasificación de las leguminosas

Existen diferentes tipos de cultivos de leguminosas con diferentes propósitos. Así, las leguminosas mayoritariamente producidas a nivel mundial son la alfalfa y el trébol, cuya principal utilización es la producción de forraje. Otras, como la soja y el cacahuete, son utilizadas para la extracción de aceites. También, las legumbres utilizadas para el consumo humano, como judías, lentejas, garbanzos, altramuces etc, constituyen otra subcategoría dentro de las leguminosas (Gutiérrez-Uribe et al., 2016). Parecen también desempeñar un considerable potencial en la biofortificación de hierro en suelos. Finalmente, cabe destacar el papel de las leguminosas en su adaptación a suelos y climas poco favorables y en la rotación de cosechas por su capacidad para fijar nitrógeno del suelo, gracias a la simbiosis con diversas bacterias radiculares (Dwivedi et al., 2015; Schipanski & Drinkwater, 2012). Dependiendo del tipo de cultivo, la capacidad de fijación del nitrógeno puede oscilar del 36% en el caso de las judías al 65% para garbanzos, lentejas o guisantes (Gutiérrez-Uribe et al., 2016).

Tabla 5: Nombre botánico y común de algunas especies de leguminosas

NOMBRE BOTÁNICO	NOMBRE COMÚN
Arachis hypogea L.	Cacahuete, maní
Cajanus cajan (L.) Millsp.	Guandú, frijol de palo, frijol chícharo, palo de gandules o quinchoncho
Cicer arietinum L.	Garbanzo, Bengal gram
Glycine max (L.) Merr.	Soja o soya
Lablab purpureus (L.) Sweet	Zarandaja, judía de Egipto, fríjol de Egipto o chaucha japonesa
Lathyrus sativus L.	Almorta, chícharo, guija, pito o tito
Lens culinaris Medik.	Lenteja
Lupinus albus L.	Altramuz, chorcho, entremozo, altramuz blanco, lupín blanco, lupino blanco o almorta
Macrotyloma uniflorum (Lam.) Verdc.	Gramo de caballo
Phaseolus lunatus L.	Pallar, garrofón, habones, judía de Lima, haba de Lima, poroto pallar o guaracaro
Phaseolus vulgaris L.	Judía común, judía negra, judía de riñón, judía pinta, habichuela, alubia, poroto, frijol
Pisum sativum L.	Guisante, arvejilla de olor
Psophocarpus tetragonolobus (L.) DC.	Frijol alado, frijol Goa, frijol de cuatro ángulos
Vicia faba L.	Haba
Vigna aconitifolia (Jacq.) Marechal	Frijol polilla, frijol mate
Vigna mungo (L.) Hopper	Fréjol negro, frijol negro, lenteja negra, judía mungo o poroto mung
Vigna radiata (L.) Wilczek	Mungo, poroto chino, loctao, soja verde, judía mungo o poroto mung
Vigna subterranea (L.) Verdc.	Judía Bambara
Vigna umbellata (Thumb.) Ohwi and Ohashi	Frijol mambé, frijol de arroz
Vigna unguiculata (L.) Walp.ssp. unguiculata	Caupí, carilla, judía de careta, frijol de carita, chíchere, chíchare, chicharillo, chícharo salvaje, fríjol chino, fríjol cabecita negra, frijol Castilla o poroto tape

Legumbres

El término legumbre procede del latín "legumen" que designa a las semillas comestibles. Por tanto, se considera legumbre seca a las semillas criadas en vainas y que son desecadas por procesos naturales para evitar su germinación, excluyéndose por ende de esta definición, las verduras como el guisante y el haba y aquellas que puedan ser utilizadas para la obtención de aceite (FAO, 2016; Hall et al., 2017; Boto Fidalgo, 2018) como la soja.

En relación a su producción a nivel mundial durante el año 2018, encontramos que la mayor producción corresponde a las alubias con (30 millones de toneladas), seguidas de los garbanzos (16 millones de toneladas) y de las lentejas (7,6 millones de toneladas). Entre los principales productores a nivel europeo destacan; en alubias, Lituania, Estonia y Polonia; en garbanzos, España, Italia y Bulgaria; y en lentejas, España y Francia (Figura 5) (FAOSTAT, 2018; MERCASA, 2019).

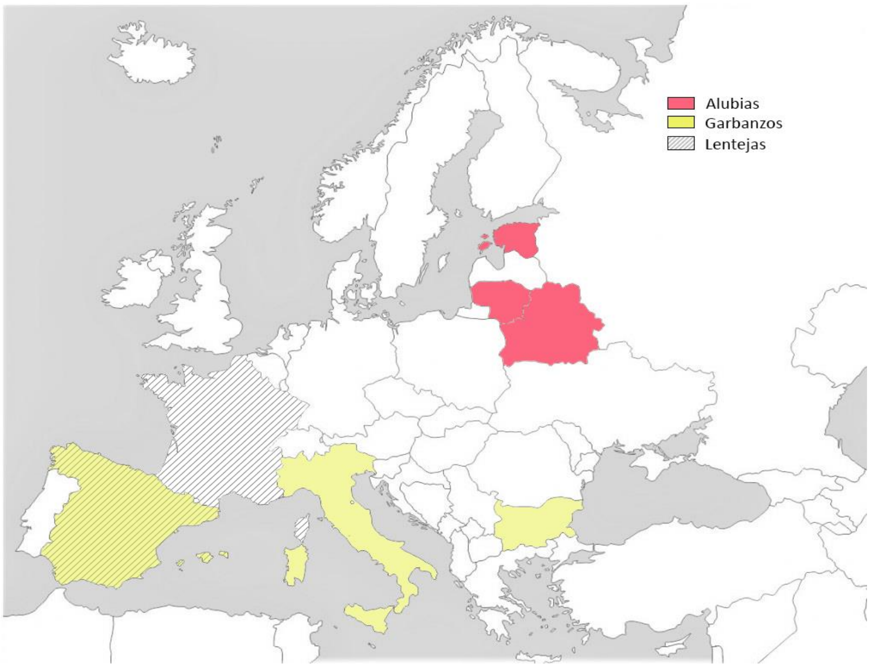


Figura 5. Principales productores de alubias, garbanzos y lentejas a nivel europeo (Fuente FAOSTAT 2018, MERCASA 2019)

En España, la producción durante el año 2018 ha aumentado un 35,49 % con respecto al año anterior (Figura 6), alcanzando alrededor de unas 136.300 toneladas. A pesar de ello, ésta resulta insuficiente para cubrir la demanda de consumo y, por lo tanto, durante el año 2018 ha sido necesario importar unas 144.700 toneladas de lentejas, garbanzos y alubias, procedentes de diversos países como Canadá, Estados Unidos, México, Argentina, China, Turquía y Portugal. Si bien es cierto que estas importaciones se han reducido (casi un 10%) con respecto al año 2017 (Mercasa, 2019).

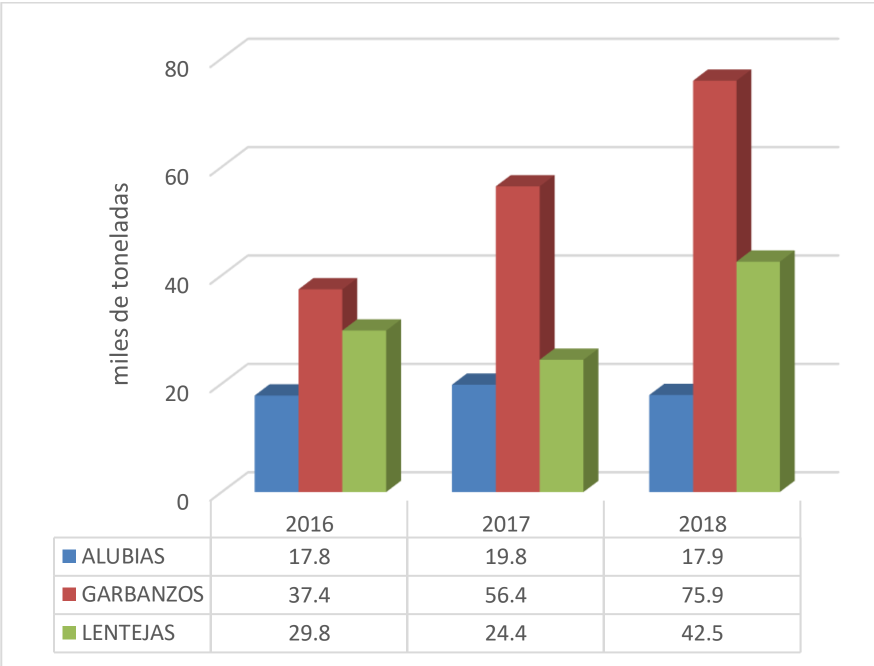


Figura 6. Producción de legumbres (lentejas, garbanzos y alubias) en España durante los años 2016-2018 (Fuente Mercasa, 2019)

En relación al consumo medio total de legumbres de la población española se establece en 13,5 g/persona/día según el Estudio ANIBES (2017). Entre las leguminosas más consumidas en España nos encontramos los garbanzos (*Cicer arietinum L.*), alubias (*Phaseolus vulgaris L.*) y lentejas (*Lens esculenta Moench o Lens culinaris Medic*). En términos per cápita, se llega a 3,2 Kg de consumo, siendo los garbanzos (1,3 kg) los más consumidos, seguidos de lentejas (1,0 kg) y alubias (0,9 kg). Sin embargo, este consumo se encuentra lejos del consumo medio mundial (en torno a 6 Kg per cápita) o el de países como India, Brasil o México que pueden superar los 10 Kg (Boto Fidalgo, 2018). También se encuentra muy alejado si lo comparamos con el consumo en España durante la década de los 60 del siglo pasado que estaba entre los 12 y 14 Kg (González-Bernal & Rubiales, 2016).

En el mercado, estas legumbres se pueden encontrar de dos formas, tanto en formato seco como en formato cocido. Debido a factores sociales, económicos y culturales, se ha producido un cambio con respecto a su consumo. La posibilidad de utilizar legumbres cocidas, listas para su uso, ha facilitado el aumento de su consumo en el hogar (Olmedilla Alonso et al., 2010) e incluso su utilización en otro tipo de preparaciones culinarias distinta del estofado (como, por ejemplo, ensaladas). Así, durante el año 2018 las ventas de legumbres cocidas se han equiparado prácticamente a la venta de legumbres secas, partiendo del hecho de que en el año 2012 las ventas de legumbres secas superaban a las cocidas en un 22% (Mercasa, 2019). Además, el cocinado no solo facilita el consumo, sino que también mejora el perfil nutricional, ya que reduce el contenido de componentes tóxicos termolábiles (factores antinutricionales) como inhibidores de tripsina, saponinas y de oligosacáridos (rafinosa, estaquiosa y verbascosa etc), manteniendo los contenidos de proteína y fibra (Naozuka & Oliveira, 2012; Vasishtha & Srivastava, 2013; Boto Fidalgo, 2018).

Valoración nutricional de las legumbres

Desde un punto de vista nutricional, las legumbres constituyen una fuente económica de proteínas (Boye et al., 2010; Los et al., 2018). En sociedades prósperas, se han utilizado como sustituto de la carne, mientras que en países de bajos ingresos, donde forman parte de dietas básicas, las leguminosas proporcionan una porción significativa de la proteína total diaria consumida, desempeñando un papel importante en el alivio de la malnutrición proteico-energética (Carvalho et al., 2012). Sin embargo, aunque contiene tantas proteínas como la carne, su calidad proteica resulta incompleta (Ramírez-Cárdenas et al., 2008; Olmedilla Alonso et al., 2010), debiendo completarse con otros alimentos ricos en metionina (cereales), complementándose y dando como resultado una proteína de gran valor (Gepts, 2001; Romero-Velarde et al., 2016).

Por otro lado, las legumbres no solo se consideran una buena fuente proteica, sino que también interesan por su contenido en carbohidratos, algunos de absorción lenta como el almidón y otros no digeribles como la fibra dietética (Tabla 6) (Tosh & Yada, 2010). Además, constituyen un producto básico en la alimentación de la población, ya que son alimentos con un bajo contenido en grasa, pero ricos en ácidos grasos esenciales (Febles et al., 2001; Zulet & Martínez, 2001).

Tabla 6: Composición nutricional media por 100 g de producto seco de las legumbres (Fuente USDA 2016)

	ENERGÍA (Kcal)	PROTEÍNAS (g)	LÍPIDOS (g)	GLÚCIDOS (g)	FIBRA (g)
ALUBIAS	337	22,33	1,50	60,75	15,30
GARBANZOS	378	20,47	6,04	62,95	12,20
LENTEJAS	352	24,63	1,06	63,35	10,70

A su vez también son una buena fuente de minerales entre los que destaca el calcio, hierro, zinc, y vitaminas del grupo B (Jodral-Segado et al., 2003; Campos-Vega et al., 2010; Hall et al., 2017). Además, se ha demostrado que poseen una serie de compuestos bioactivos (compuestos fenólicos, saponinas, taninos), con numerosos beneficios para la salud (López-Martínez et al., 2017; Giusti et al., 2019). También cabe destacar su papel durante la infancia, siendo una excelente fuente de proteínas vegetales. Durante esta etapa (a partir de los 10 - 12 meses), es importante proporcionar la cantidad recomendada de proteínas, pudiendo utilizar estas legumbres como dieta complementaria a la lactancia materna (Pavón et al., 2007). También han sido empleadas en fórmulas infantiles (Vioque et al., 2001), ya que dicha aplicación supone una mejora nutricional y funcional (Sarmento, 2012).

Como consecuencia de todo lo anterior la Organización Mundial de la Salud (OMS) recomienda un consumo de legumbres de al menos 3 veces por semana, ya que ayudaría a reducir el riesgo de enfermedades asociadas a la alimentación como obesidad, hipercolesterolemia, diabetes mellitus, estreñimiento, diverticulitis, cáncer de colon, entre otras (Kalogeropoulos et al., 2010; Olmedilla Alonso et al., 2010; Los et al., 2018). También, debido a su deficiencia en aminoácidos azufrados como metionina, se recomienda emplearlas en dietas vegetarianas, como complemento de cereales, compensando la escasez de estos últimos en lisina. Sin embargo, aunque los expertos advierten sobre los efectos beneficiosos de su consumo (USDA,2016b), a día de hoy existe una cierta controversia entre esta concienciación y su consumo (Kalogeropoulos et al., 2010; FEN, 2017). Una de las causas en el descenso del consumo de legumbres es el aumento del nivel de vida en los países desarrollados que ha producido una modificación de los patrones dietéticos de los consumidores, incrementándose la ingesta de proteínas de origen animal en detrimento de las proteínas de origen vegetal.

Por ello, el año 2016 se proclamó como el Año Internacional de las legumbres "International year of Pulses -IYP 2016" (FAO, 2016a), con el propósito de aumentar la concienciación sobre los beneficios nutricionales de estos alimentos de origen vegetal (Ramírez-Ojeda et al., 2018).

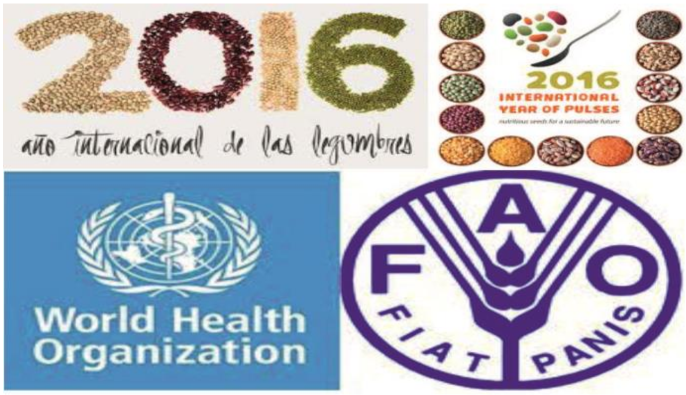


Figura 7. Logo conmemorativo del año de las leguminosas

No obstante, como ya se ha comentado, también debemos destacar que las legumbres poseen una serie de sustancias consideradas como antinutritivas, entre las que encontramos ácido fítico, oxalatos… entre otras, las cuales pueden afectar a la digestibilidad y bioaccesibilidad de nutrientes (Olmedilla Alonso et al., 2010; Los et al., 2018). Para paliar los efectos negativos de estos compuestos, generalmente previo a su consumo las legumbres se someten a una serie de procesos tecnológicos y/o culinarios como el remojado, tratamiento térmico, germinado, etc (Rachwa-Rosiak et al., 2015). Estos procesos posibilitan la eliminación de estos componentes no nutritivos no deseables presentes en las legumbres crudas, aumentando así la biodisponibilidad del resto de nutrientes presentes en su composición y por tanto mejorando su valor nutricional (Faria et al., 2018).

1.3.1. ALUBIAS

La alubia (*Phaseolus vulgaris* L.), conocida comúnmente como judía, es originaria de América, y a nivel mundial es la especie más cultivada (Olmedilla Alonso et al., 2010). Planta anual, que posee diferentes nombres dependiendo de la región española en la que nos encontremos: alubias, fabes, mongetes, bajocas, caparrones, habichuelas, fréjoles. Su semilla presenta forma de riñón y diversos colores, que dependerá de la variedad (Mamidi et al., 2011). En España podemos encontrar principalmente 3 especies que son Judía Común

(*Phaseolus vulgaris),* judía de España o Judía escarlata (*Phaseolus multiflorus*), y Judía de Lima o Garrafó (*Phaseolus lunatus*). La mayor parte de las judías cultivadas en España pertenecen al primer tipo.

Dentro de la Judía Común encontramos distintas variedades como *Alubia blanca de riñón y Alubia Blanca Redonda*, conocida popularmente como "manteca" de grano blanco, lleno, redondo y tamaño grande. De vaina verde y flor blanca. Su zona ideal de cultivo es La Bañeza (León) y su precio popular, unido a su buena calidad, las hacen muy valoradas; *Caparrón*, de tamaño mediano y de color rojo con fondo blanco. Se producen, sobre todo en La Rioja. Es el ingrediente principal de la olla podrida en Anguilano (Burgos); *Tolosana,* sus hojas y vainas son de color verde oliva y la flor clara. El grano es ligeramente elíptico, casi redondo y lleno, de tamaño mediano y color rojo oscuro. Es muy apreciada en el País Vasco y se cree que procede de Tolosa (Guipúzcoa). En España es bastante utilizada en potajes, aunque la calidad proteica es baja; *Negrilla*, de color negro y con gran cantidad de fécula y *Palmeña jaspeada* de color vino tinto sobre fondo rosado, alargada y grande. Se parece mucho a la *Pinta de León*, aunque ésta es más pequeña y redonda. Se consume principalmente en Cantabria y en el País Vasco, aunque se produce en León.

Dentro de la especie *Phaseolus lunatus* encontramos el Garrafó, vulgarmente conocida como garrofa o garrofón. La vaina es corta y ancha y tiene numerosas flores blancas sobre pedúnculos. El grano es aplanado y arriñonado con estrías radiadas. Es mantecosa y grande (similar al tamaño de la fabe asturiana), y puede ser blanca o verdosa. Se cultiva en la Comunidad Valenciana donde se utiliza en la elaboración de la paella. Destaca su bajo contenido en grasa y es muy rica en hierro.

Finalmente, la especie *Phaseolus multiflorus* tiene una variedad ampliamente conocida en España como Judión de la Granja, vulgarmente llamada Judión. Las hojas y las vainas son verdes oscuras y posee gran cantidad de flores blancas y lilas. El tamaño es muy grande y los granos pueden ser blancos, negros o jaspeados. Son famosas las de La Granja en Segovia, pero se producen muy pocas. También se cultivan en Ávila. Tienen un alto contenido en grasa (Ministerio de Agricultura, Pesca y Alimentación, 2018).

Con todo lo anterior en España existen diferentes Indicaciones Geográficas Protegidas (IGP) y/o Denominaciones de Origen Protegidas (DOP) para este tipo de legumbres (Figura 8), las cuales confieren un valor añadido en calidad y precio a este tipo de alimento. Las principales características morfológicas y organolépticas se detallan a continuación.

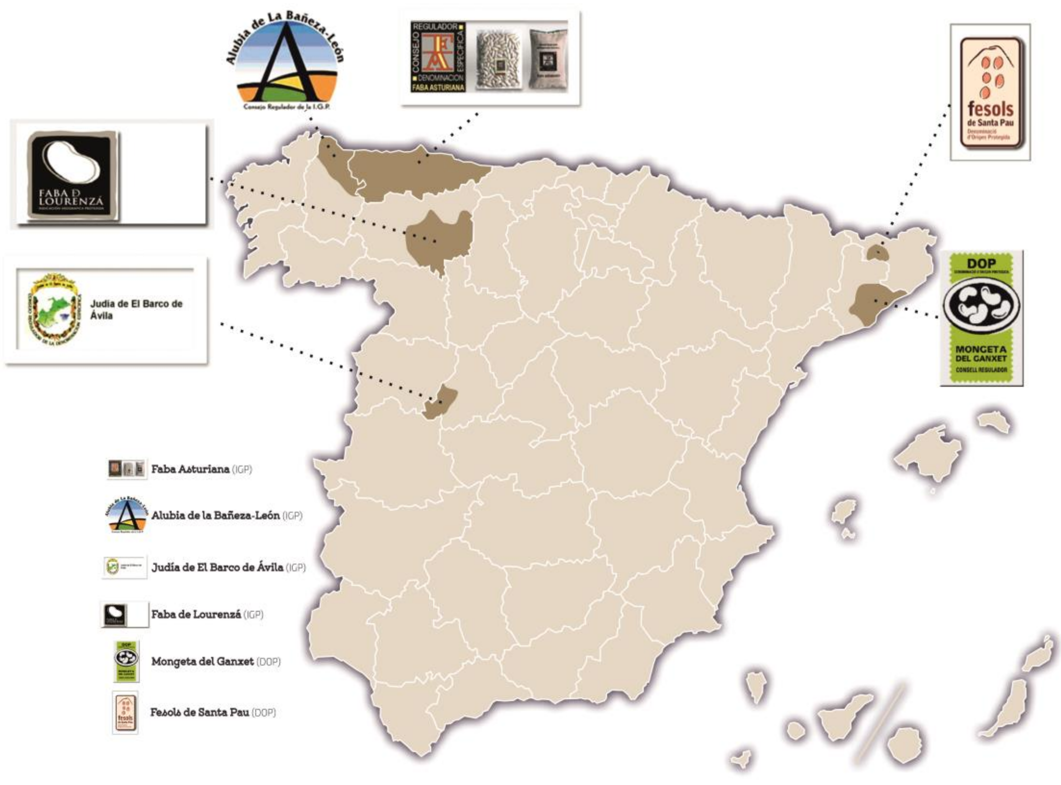


Figura 8. Alubias con Denominación de Origen e Indicaciones Protegidas en España

Denominaciones de Origen e Indicaciones Protegidas

I.G.P. Faba Asturiana. Judía de la variedad Granja Asturiana, de color blanco cremoso, de forma arriñonada, larga y aplanada y tamaño grande, unos 100 - 110 granos por 100g de semillas. La zona de producción está constituida por los terrenos ubicados en el territorio de la

Comunidad Autónoma del Principado de Asturias. Entre otros platos, se utiliza en la fabada asturiana, el grano se presenta entero, con piel lisa, albumen blando y mantecoso y poco o nada harinoso.

I.G.P. Alubia de la Bañeza-León. La zona de producción agrícola se encuadra en 98 municipios de la provincia de León pertenecientes a las comarcas agrarias de Astorga, El Páramo, Esla-Campos, La Bañeza, La Cabrera y Tierras de León, así como en 20 municipios de la comarca de Benavente-Los Valles, en la provincia de Zamora, colindante con la anterior. Utiliza las variedades Canela de León, Plancheta o planchada, Pinta Riñón y Manteca de León.

IGP Judía de El Barco de Ávila. Pertenecen a esta IGP las variedades blanca riñón, blanca redonda, arrocina, blanca planchada, morada redonda, morada larga y judión de El Barco.

I.G.P. Faba de Lourenzá. Conocida como "Faba Galaica", de color blanco uniforme, sin dibujos, de tamaño muy grande y forma arriñonada, larga y semillena. Se caracteriza por la escasa proporción de piel (entre 8 - 10 %) y su elevada capacidad de absorción de agua (superior al 100 %). Tal y como indica su nombre su principal zona de producción se encuentra localizada en la comarca de Lourenzá (Lugo).

D.O.P. Mongeta del Ganxet. De grano blanco, ligeramente brillante, aplanado y fuertemente arriñonado. Su nombre significa "Ganxet" pequeño gancho en catalán. Su peso oscila entre 40-50g por 100g de semillas. A nivel organoléptico se caracterizan por piel ligeramente rugosa y muy poco perceptible y elevada-persistente cremosidad.

D.O.P. Fesols de Santa Pau. Ampara las semillas secas, cocidas y en conserva de las variedades tradicionales Tavella Brisa, Setsetmanera y Gra Petit. La zona geográfica de producción y elaboración corresponde a los municipios de Santa Pau (principal núcleo de producción), Castellfollit de la Roca, Les Planes d'Hostoles, Les Preses, Olot, Sant Feliu de

Pallerols y Sant Joan les Fonts (pertenecientes a la comarca de la Garrotxa, en la Comunidad Autónoma de Cataluña).

1.3.2. GARBANZOS

El garbanzo, conocido desde la más remota antigüedad, es originario de Turquía. Se extendió hacia Europa y más tarde a los continentes de África, América y Oceanía (Lev-Yadun et al., 2000). En España, su cultivo y consumo fue incentivado por los cartagineses (570 – 206 a. de C), siendo en muchas ocasiones la base de su dieta. Es una planta anual, cuya siembra se realiza en otoño en las zonas templadas y en febrero en las más frías, perteneciente a la especie *Cicer Arietinum*, L., de tallos cuadrangulares y flores blancas o azuladas y frutos ovoides que contienen dos frutos (Rachwa-Rosiak et al., 2015). Los granos son redondeados o rugosos de color amarillento. La variación morfológica y de color viene marcada por las diferencias del medio y las condiciones climatológicas en cada cosecha.

Según la forma de la semilla, el color y en menor medida el tamaño, podemos encontrar 3 grupos principales: *`desi´, `kabuli´ y `guisante o gulabi´* esta última de poca importancia comercial (Knights & Hobson, 2016). Con respecto a su forma, la especie *desi* tiene una forma angular y un recubrimiento grueso y coloreado (principalmente marrón), mientras que las semillas *kabuli* tienen una forma más redondeada y su cubierta es delgada, de color blanco a beige crema. Finalmente, *gulabi*, tiene un tamaño pequeño, liso y color también claro (Agriculture and Agri-Food Canada, 2008).

Con respecto a su consumo en España, los garbanzos se encuentran entre las legumbres más consumidas (Alajaji & El-Adawy, 2006; Mercasa, 2017). Todos los tipos de garbanzos cultivados en nuestro país pertenecen a la especie *Cicer Arietinum* L. Los principales tipos son: *Castellano,* el más consumido del país. De color amarillento, tamaño medio o grande, forma esférica, superficie bastante lisa y pico curvo muy pronunciado. Se cultiva tanto en Andalucía como en la Meseta Central. Dentro de esta variedad encontramos un ecotipo de calidad, el garbanzo de Fuentesaúco (Zamora); *Blanco lechoso*, muy apreciado por los consumidores. Su grano es de color blanco amarillento, grueso y alargado, achatado por los lados de forma irregular y marcados surcos. Su cultivo se centra, especialmente, en Andalucía y Extremadura; *Venoso andaluz*, de tamaño grueso, algo alargado y la superficie recorrida por líneas de color más claro. Es bastante rústico y de sabor fuerte. Es el de mejor calidad proteica y tiene gran contenido en hierro y calcio. Se cultiva en Andalucía, concretamente en Granada; *Chamad*, obtenido de una hibridación con la variedad conocida como castellano. De tamaño más grande y claro que el castellano, su forma es irregular. Ocupa el segundo lugar en calidad proteica. Es típico también de Andalucía, cultivándose preferentemente en Granada; *Pedrosillano*, apreciado por los consumidores, que destacan sus cualidades organolépticas. Es de tamaño más pequeño y de forma muy redondeada, tegumento liso y línea de separación de los cotiledones bien marcada. De color naranja-amarillento es característico por su pequeño y agudo pico. Requiere de más cocción. Se cultiva en Salamanca, en Pedrosillo el Ralo en la zona de la Armuña (Mercasa, 2017).

Denominaciones de Origen e Indicaciones Protegidas

Al igual que ocurría para las alubias, en España existen diferentes Denominaciones de Origen e Indicaciones Protegidas para esta legumbre (Figura 9).

I.G.P. Garbanzo de Fuentesaúco. Se caracteriza por su tamaño, entre mediano y grande, su pico curvo y pronunciado y una piel de rugosidad intermedia. Tras la cocción, los garbanzos se mantienen íntegros, la piel es blanda y suave al paladar y su interior uniforme y mantecoso.

I.G.P. Garbanzo de Escacena. El producto que ampara esta Indicación Geográfica Protegida es el fruto de la especie vegetal *Cicer arietinum* L., del ecotipo local del Campo de Tejada y de las variedades registradas o que se registren del tipo comercial Lechoso. Cultivado en espacio de campiña entre las provincias de Huelva y Sevilla, abarcando poblaciones como La Palma del Condado, Sanlúcar la Mayor, Olivares, etc. De color blanco amarillento muy claro y forma alargada y achatada por los lados, presentando irregularidades en su superficie con profundos surcos y abultamientos.

Figura 9. Garbanzos con Denominación de Origen e Indicaciones Protegidas en España

1.3.3. LENTEJAS

Las lentejas que reciben el nombre científico de *Lens culinaris,* son originarias de los países del sudoeste de Asia. Junto a los cereales se cree que fue uno de los primeros cultivos, existiendo datos desde el año 6600 a. de C (FEN, 2017). Estas semillas procedentes de planta anual herbácea, pertenecientes a la familia de las *Papilionáceas*, y tienen forma de platillo. Es una planta fácil de cosechar, cultivada en regiones templadas (Dhuppar et al., 2012) que posee tallos de 30 a 40 cm, endebles, ramosos y estriados, hojas oblongas. Sus flores son de color blanco, azul claro o moradas, sobre un pedúnculo axilar, y fruto en vaina pequeña, con dos o tres semillas pardas en forma de disco de medio centímetro de diámetro aproximadamente. En relación a su color, las semillas pueden variar desde amarilla a anaranjadas, verde, marrón o negras (Joshi et al., 2017).

En España hay principalmente tres variedades de *Lens Culinaris*: “*Vulgaris”*, “*Variabilis”* y “*Dupuyensis”*.

Dentro de la variedad *Vulgaris* encontramos la *Rubia Castellana*, denominada también, lentejón o lenteja de la reina. De color verde claro con tonalidades decoloradas se oscurece con el paso del tiempo. Su tamaño, superior a otros tipos españoles, es de 6 a 8 mm de diámetro. Se cultiva preferentemente en Castilla - La Mancha, Salamanca y Granada; *Rubia de Armuña*, que puede considerarse una diferenciación de la anterior. Recibe también el nombre de Gigante de Gomecello. De color verde claro y a veces jaspeado, su tamaño puede alcanzar los 9 mm. Su cultivo se centra en el norte de Salamanca.

De la variedad *Variabilis* encontramos la *Pardina* se denominan también Franciscanas o Francesas. Se caracteriza por su color pardo, marrón o pardo rojizo. Es de mediano tamaño entre 4 y 5 mm. Rica en hidratos de carbono, adecuada para combinar con pasta y ensalada. Debe cocerse a fuego muy lento. Se cultiva en tierra de Campos, León, Palencia y Burgos. De sabor agradable y suave al paladar, ocupa el segundo lugar respecto a su calidad proteica (por detrás de la de Armuña).

Finalmente, dentro de la variedad *Dupuyensis* nos encontramos la *Verdina*. El tamaño del grano es pequeño (de 3 a 4 mm), su color varía entre el verde y el verde amarillento con manchas oscuras. Su sabor es muy agradable, utilizándose en la elaboración de purés y cremas. Se cultiva en Valladolid, León, Palencia y Burgos (Ministerio de Agricultura, Pesca y Alimentación, 2018).

Denominaciones de Origen e Indicaciones Protegidas

También para esta legumbre, existen diferentes Denominaciones de Origen e Indicaciones Protegidas en España (Figura 10).

<u>IGP Lenteja de La Armuña</u>. Es una de las más sabrosas y originales del mundo y cuenta con una variedad propia e inimitable.

<u>IGP Lenteja de la Tierra de Campos</u>. Una de las más aptas para cocinar, ya que ni siquiera necesita remojo. Se caracteriza por su color pardo o marrón rojizo. De pequeño tamaño, es apreciada por su blanda textura.

Figura 10. Lentejas con Denominación de Origen e Indicaciones Protegidas en España

Conservación, preelaboración y aplicaciones gastronómicas de las legumbres

Las legumbres son expedidas en diferentes presentaciones: secas, en remojo, cocidas, y envasadas. Cuando se compran a granel (secas) deben estar enteras, sin olor y con un color uniforme.

Perteneciente a su conservación cabe destacar que, aunque debemos consumirlas lo antes posible, una legumbre en su formato seco aguanta entre 9 y 12 meses. Deben almacenarse en lugares secos y siempre en recipientes cerrados. También pueden conservarse congeladas una vez cocidas. Los botes cerrados herméticamente que podemos encontrarnos en el mercado, una vez abiertos deberemos conservar en la nevera solo durante unos días El proceso de preelaboración incluye la eliminación de impurezas y granos defectuosos y el remojado, que para el caso de las alubias deben ponerse en remojo con agua fría el día anterior (mínimo unas 12 horas) previo a su proceso de cocción y en el caso de los garbanzos deberemos ponerlos en agua tibia y una pequeña cantidad de sal gorda por espacio entre doce y veinticuatro horas, dependiendo del tipo y el tamaño de grano. Se deben lavar previo a su cocción especialmente si durante el remojado se ha empleado bicarbonato. Las lentejas al igual que las alubias se pondrán en remojo con agua fría. Sin embargo, dependiendo del tiempo transcurrido desde su recolección, no todas las lentejas requerirán de remojado previo (FEN, 2017).

El tiempo de cocción dependerá de varios factores como pueden ser la dureza del agua, la intensidad del fuego, el remojado, así como tanto la variedad como el tiempo que tienen las legumbres, pudiendo ir desde una hora hasta las 3 horas. Respecto a su cocción, las alubias deberán cocinarse partiendo desde agua fría y no sazonar al principio ya que, junto con la cal del agua, se crea una película alrededor de la legumbre endureciéndola y puede provocar que estas se abran al perder el hollejo. En el caso del garbanzo, al contrario que el resto de las legumbres, se ponen a cocer en agua tibia con un pellizco de sal, ésta debe ser uniforme y se debe añadir siempre agua caliente, para que no se rompa el hervor, ya que un cambio en la temperatura del líquido de cocción podría hacer que se encallasen, con lo cual permanecerían duros tras la cocción. Con respecto a la cocción de las lentejas, estas se deben poner a hervir en recipiente de material inalterable, partiendo desde agua fría con elementos aromáticos (FEN, 2017).

En relación a su consumo, existen numerosos platos a base de alubias. Así en España encontramos varios platos típicos como la fabada asturiana, alubias con chorizo, judías en ajo colorado, cocido vasco, escudella catalana (Escudella i carn d´olla), pote gallego, pote asturiano, mochetas con butifarra, cocido montañés. También podemos encontrar unas cremas que se elaboraban tradicionalmente, de modo que, cambiándoles la forma, se aprovechaban las sobras. Un ejemplo de ello es la crema bretona elaborada con judía blanca y la crema conde con alubia roja.

Los garbanzos pueden comerse cocidos, tostados, fritos e incluso en forma de harina, aunque ha sido consumida en algunas zonas también como infusión, en una especie de café, una vez que los garbanzos han sido tostados y molidos. En la cocina española posee su posición como ingrediente en diversos platos como lo es el cocido madrileño, cocido Andaluz o Extremeño, cocido maragato (típico de León), los callos a la andaluza, Potaje de Vigilia etc. El garbanzo es muy común en la cocina de la india y se emplea en numerosos platos, formando parte de las legumbres denominadas Dal (término utilizado para denominar a las legumbres a las que se les ha retirado la piel, usado muy comúnmente en el sur de Asia) y frecuentemente en la forma de harina. En la cocina del Magreb es muy peculiar una especie de pasta de garbanzos denominada hummus.

En el caso de las lentejas, estas pueden ser consumidas de muy diferentes formas: como plato principal como pueden ser los potajes, en sopas, o purés, (enteras o combinadas con vegetales o cereales) o en ensaladas. Suelen tomar el nombre del género al que acompañan por ejemplo lentejas con chorizo, con oreja etc. Platos internacionales son las lentejas lionesa y la crema Essaú.

1.4. DESCRIPCIÓN DE LAS MUESTRAS ANALIZADAS

1.4.1 Leguminosas

Coincidiendo con el Año Internacional de las Legumbres (2016), mediante el cual se propuso sensibilizar a la opinión pública sobre las ventajas nutricionales de este grupo de alimentos como parte de una alimentación sostenible, encaminada a lograr la seguridad alimentaria y la nutrición, se decidió realizar como objetivo principal de mi Tesis una valoración nutricional de las tres legumbres más consumidas en España (garbanzos, judías y lentejas). Para ello se seleccionaron los dos tipos de formatos en los que se comercializan (secas o ya cocidas, listas para su consumo).

En una primera aproximación, se contactaron con diferentes empresas productoras y comercializadoras para que nos facilitaran las muestras. Ante la escasa respuesta obtenida se realizó un muestreo comprando directamente las legumbres en supermercados o centros comerciales. Para ello, seleccionamos tres marcas de reconocido prestigio y habitualmente presentes en supermercados a lo largo de la geografía española. Se procuró además que la localización geográfica de las empresas fuera diferente. Las marcas seleccionadas fueron Luengo, La Asturiana y Hacendado. Para obtener una muestra suficientemente representativa el muestreo se realizó en tres periodos diferentes (de septiembre de 2015 a marzo de 2016).

1.4.2 Potitos ecológicos

Coincidiendo con el periodo de realización de mi tesis doctoral, se firmó un contrato entre un grupo de investigación del Departamento de Bromatología y Tecnología de Alimentos y la empresa Bioalimentación Infantil de Andalucía SL. Dicha empresa, dedicada a la fabricación de alimentos infantiles con ingredientes ecológicos, tenía por objetivo determinar en los alimentos por ella fabricados parámetros microbiológicos (determinación de microorganismos patógenos y desarrollo de modelos predictivos de crecimiento microbiano) así como desarrollar estudios de vida comercial, análisis sensorial y valoración nutricional.

En relación a este último objetivo, se contactó con el grupo AGR – 013 "Calidad Agroalimentaria y Nutrición" en el que yo he desarrollado mi tesis para que realizara la valoración nutricional de estos potitos realizados con ingredientes ecológicos (determinación de principios inmediatos, análisis de elementos inorgánicos y su bioaccesibilidad), contando conmigo como analista. Fue así como se decidió incorporar los datos obtenidos en esta investigación a mi Tesis doctoral como otro de sus principales objetivos.

Los ingredientes utilizados en la elaboración de cada una de las recetas analizadas se muestran en la siguiente Tabla.

Tabla 7. Ingredientes utilizados en la elaboración de cada una de las recetas analizadas.

	CÓDIGO	INGREDIENTES
Plátano, Pera, Granada	PPG	Fruta 97,5% (plátano, pera, zumo de granada, zumo de limón), Harina 2,5%, Vitamina C.
Verduras, Garbanzos, Pera	GVP	Agua de cocción, Verduras y Hortalizas 45,5% (Patata, Calabaza, Tomate, Cebolla, Judías verdes), Garbanzos 6%, Pera 3%, Harina 2,5%, Aceite de oliva virgen extra 2%, Pimentón dulce 0,14% y Especies.
Ternera, Verduras	TEV	Verduras y Hortalizas 67,5% (Tomate, Patata, Cebolla, Guisantes, Zanahoria), Carne de ternera 12%, Agua de Cocción, Harina 2,5%, Aceite de oliva virgen extra 2% y Especies.
Plátano, Manzana, Naranja	PMN	Frutas 100% (plátano, manzana, zumo de naranja, zumo de limón), vitamina C
Multifrutas	MFR	Fruta 95.5% (plátano, manzana, zumo de naranja, pera, zumo de uva, zumo de limón), zumo de zanahoria 4.5%, vitamina C.
Crema de Calabacín	CCA	Verduras y Hortalizas 75% (Calabacín, Patata, Puerro, Cebolla), Agua de cocción, Aceite de oliva virgen extra.
Calabaza, Manzanilla	CM	Agua de cocción, Verduras y Hortalizas 69,5% (Calabaza, Patata, Cebolla, Puerro), Aceite de oliva virgen extra 2%, Hierbabuena y Flor de manzanilla.
Crema de Verduras Variadas	CVV	Agua de cocción, Verduras y Hortalizas 72% (Patata, Puerro, Judías verdes, Cebolla, Calabacín) y Aceite de oliva virgen extra 2%.
Pollo, Verduras	POV	Agua de Cocción, Verduras y Hortalizas 56% (Patata, Tomate, Cebolla, Pimiento Rojo), Carne de Pollo 12%, Aceite de oliva virgen extra 2%, Perejil y Especies
Pasta, Pollo, Verduras	PPOV	Agua de cocción, Verduras y Hortalizas 51% (Patata, Tomate Cebolla), Carne de pollo 12%, Pasta Integral 4%, Aceite de oliva virgen extra 2% y Especias

Todas las muestras (tanto de legumbres como de potitos ecológicos) fueron liofilizadas a su llegada al laboratorio (Scanvac Cool Safe freeze dryer, model 55-44) y posteriormente homogeneizadas en un mortero cerámico. Las muestras fueron almacenadas y envasadas en bolsas de vacío hasta el momento de su análisis.

Los estudios realizados sobre los dos grupos de muestras alimentarias que acabamos de describir se estructuran en los siguientes capítulos de mi Tesis Doctoral por compendio de publicaciones, cada uno de los cuales se corresponde con un artículo científico o bien un capítulo de libro.

CAPÍTULO 1

En el Capítulo 1, se realiza una extensa revisión bibliográfica sobre dos leguminosas: guisantes y lentejas. Este capítulo se realiza por invitación de la editorial Elsevier a contribuir en la publicación del libro Encyclopedia of Food and Health. Se decidió incluir parte del contenido de la revisión bibliográfica realizada para esta Tesis como capítulo de este libro. En dicha revisión, se aborda el valor nutricional de estas dos variedades de leguminosas, tanto su contenido en macronutrientes (carbohidratos, proteínas, lípidos y fibra) como micronutrientes (vitaminas, minerales y elementos traza). Además de ello, se presentan los posibles efectos beneficiosos para la salud derivados de su consumo. Por otro lado, también se describen la presencia de ciertos factores antinutricionales (inhibidores de proteasas e inhibidores de α-amilasa) que pueden reducir su calidad nutricional, así como las diferentes metodologías empleadas (procesos de remojo, germinación, fermentación, cocción y el uso de ciertas enzimas) con el propósito de reducir o eliminar estos factores antinutricionales. Como ya se ha comentado, este capítulo (Peas and Lentils) fue publicado en el libro Encyclopedia of Food and Health 1st Edition de la Editorial Elsevier.

CAPÍTULO 2

En el Capítulo 2, realizamos una valoración nutricional de las tres variedades de leguminosas más consumidas en España (alubias, garbanzos y lentejas) tanto en formato seco como en formato cocido (listo para su consumo). El estudio refleja el análisis de los minerales y elementos traza (Ca, Mg, Fe, Zn, Cu y Mn), y su bioaccesibilidad (solubilidad y dializabilidad) así como la determinación de su contenido de proteínas y grasas. La influencia de estos factores dietéticos sobre el contenido y bioaccesibilidad de los elementos inorgánicos es también analizada. Finalmente, se desarrolla un modelo probabilístico (con una herramienta estadística como @Risk) sobre las contribuciones a las Ingestas Dietéticas de Referencia para los elementos inorgánicos estudiados a partir del consumo de estas leguminosas. Esta evaluación es muy importante teniendo en cuenta que el año 2016 fue clasificado por la ONU como "Año Internacional de las Leguminosas". Este artículo (Mineral and trace element content in legumes (lentils, chickpeas and beans): Bioaccesibility and probabilistic assessment of the dietary intake) fue publicado en la revista Journal of Food Composition and Analysis.

CAPÍTULO 3

En el Capítulo 3, se presenta una valoración nutricional exhaustiva de diez formulaciones de potitos para alimentación infantil, con diferentes ingredientes y categorizados con el atributo de ecológicos. Esta valoración comprende la determinación de los minerales y oligoelementos nutricionalmente más relevantes (Ca, Mg, Fe, Zn, Cu y Mn), así como la determinación de su bioaccesibilidad (solubilidad y dializabilidad) obtenida mediante un modelo de digestión gastrointestinal *in vitro* en el que se reproducen las condiciones que tienen lugar en el aparato digestivo humano. Del mismo modo, también se determinó el contenido de proteína y grasa en los potitos ecológicos, así como la influencia que tienen estos macronutrientes sobre la bioaccesibilidad de los elementos inorgánicos anteriormente estudiados. Por último, se utilizó un modelo probabilístico para evaluar la contribución de los micronutrientes inorgánicos presentes en los potitos ecológicos a las Ingestas Dietéticas de Referencia para población infantil. Los modelos se desarrollaron a partir de valores de contenido total y bioaccesible de los oligoelementos. Este artículo (Influence of dietary components on minerals and trace elements bioaccessible fraction in organic weaning food: a probabilistic assessment fue publicado en la revista European Food Research and Technology.

CAPIÍTULO 4

En el Capítulo 4, siguiendo la misma metodología de los capítulos anteriores, se analiza si los potitos ecológicos estudiados presentan o no un menor contenido en un metal pesado como el cadmio frente a los convencionales (descritos en la bibliografía). También se estudia su contenido en un micronutriente antioxidante como el selenio y el efecto protector de este último frente a la toxicidad del cadmio. Se han analizado tanto el contenido total de cadmio y selenio como su bioaccesibilidad, así como la influencia sobre ambas variables de otros factores dietéticos (proteínas, grasa y fibra) presentes en las formulaciones. Por último, se expone una evaluación de las contribuciones a DRI o PTWI de Se y Cd derivadas del consumo de estos alimentos utilizando para ello un enfoque estadístico novedoso como @Risk. Los modelos se desarrollaron a partir de valores de contenido total y bioaccesible (dializable) de los oligoelementos. Este artículo (Selenium and cadmium in bioaccessible fraction of organic weaning food: Risk assessment and influence of dietary components) fue publicado en la revista Journal of Trace Elements in Medicine and Biology.

Bibliografía

AAVV. (2013). *Science/AAAS | Special Issue: Smarter Pest Control*. https://www.sciencemag.org/site/special/pesticides/index.xhtml

Abrams, S. A. (2013). Zinc for preterm infants: Who needs it and how much is needed? 1-3. *The American Journal of Clinical Nutrition*, *98*(6), 1373–1374. https://doi.org/10.3945/ajcn.113.076489

Aertsens, J., Verbeke, W., Mondelaers, K., & van Huylenbroeck, G. (2009). Personal determinants of organic food consumption: A review. *British Food Journal*, *111*(10), 1140–1167. https://doi.org/10.1108/00070700910992961

Agarwal, S., Zaman, T., Murat Tuzcu, E., & Kapadia, S. R. (2011). Heavy metals and cardiovascular disease: Results from the national health and nutrition examination survey (NHANES) 1999-2006. *Angiology*, *62*(5), 422–429. https://doi.org/10.1177/0003319710395562

Agostoni, C., Decsi, T., Fewtrell, M., Goulet, O., Kolacek, S., Koletzko, B., Michaelsen, K. F., Moreno, L., Puntis, J., Rigo, J., Shamir, R., Szajewska, H., Turck, D., van Goudoever, J., & ESPGHAN Committee on Nutrition. (2008). Complementary Feeding: A Commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, *46*(1), 99–110. https://doi.org/https://doi.org/10.1097/01.mpg.0000304464.60788.bd

Agriculture and Agri-Food Canada. (2008). *An overview of the Canadian Agriculture and Agri-Food system*. Agriculture and Agri-Food Canada.

Agte, V., Jahagirdar, M., & Chiplonkar, S. (2005). Apparent absorption of eight micronutrients and phytic acid from vegetarian meals in ileostomized human volunteers. *Nutrition*, *21*(6), 678–685. https://doi.org/10.1016/j.nut.2004.11.007

Alajaji, S. A., & El-Adawy, T. A. (2006). Nutritional composition of chickpea (Cicer arietinum L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis*, *19*(8), 806–812. https://doi.org/10.1016/j.jfca.2006.03.015

Alcázar Arroyo, R., Albalate Ramón, M., de Sequera Ortiz, Pa., Corchete Prats, E., Puerta Carretero, M., & Ortega Díaz, Ma. (2011). *Algoritmos en nefrología. Módulo. 01: Trastornos hidroeletrolíticos y del equilibrio ácido-base*. Grupo Editorial Nefrología de la Sociedad Española de Nefrología.

Amaro López, M. Á., & Cámara Martos, F. (2004). Iron availability: An updated review. *International Journal of Food Sciences and Nutrition*, *55*(8), 597–606. https://doi.org/10.1080/09637480500085820

Anderson, J. J. B. (2004). Minerals. In *Food, nutrition, and diet therapy* (11 th edn, pp. 135–143). In: Mahan LK, Escott-Stump S (eds). http://www.ars.usda.gov/fnic/foodcomp

Andiarena, A., Irizar, A., Molinuevo, A., Urbieta, N., Babarro, I., Subiza-Pérez, M., Santa-Marina, L., Ibarluzea, J., & Lertxundi, A. (2020). Prenatal manganese exposure and long-term neuropsychological development at 4 years of age in a population-based birth cohort. *International Journal of Environmental Research and Public Health*, *17*(5). https://doi.org/10.3390/ijerph17051665

Angood, K. M., Wood, J. D., Nute, G. R., Whittington, F. M., Hughes, S. I., & Sheard, P. R. (2008). A comparison of organic and conventionally-produced lamb purchased from three major UK supermarkets: Price, eating quality and fatty acid composition. *Meat Science*, *78*(3), 176–184. https://doi.org/10.1016/j.meatsci.2007.06.002

Araya, M., Koletzko, B., & Uauy, R. (2003). Copper Deficiency And Excess In Infancy: Developing A Research Agenda. *Journal of Pediatric Gastroenterology and Nutrition*, *37*(4), 422–429. https://doi.org/https://doi.org/10.1097/00005176-200310000-00005

Arnon, S., Regev, R. H., Bauer, S., Shainkin-Kestenbaum, R., Shiff, Y., Bental, Y., Dolfin, T., & Litmanovitz, I. (2009). Vitamin E levels during early iron supplementation in preterm infants. *American Journal of Perinatology*, *26*(5), 387–392. https://doi.org/10.1055/s-0029-1214233

Arredondo, M., Martínez, R., Núñez, M. T., Ruz, M., & Olivares, M. (2006). Inhibition of iron and copper uptake by iron, copper and zinc. *Biological Research*, *39*, 95–102. https://doi.org/https://doi.org/10.4067/s0716-97602006000100011

Arvedson, J. (2006). Swallowing and feeding in infants and young children. In *GI Motility online* (Vol. 9, pp. 1–20). Nature Publishing Group. https://doi.org/10.1038/gimo17

Aschner, J. L., & Aschner, M. (2005). Nutritional aspects of manganese homeostasis. *Molecular Aspects of Medicine*, *26*(4–5), 353–362. https://doi.org/10.1016/j.mam.2005.07.003

ATSDR. (2012). *TOXICOLOGICAL PROFILE FOR MANGANESE*.

Avila, D. S., Gubert, P., Roos, D. H., Puntel, R., & Aschner, M. (2016). Manganese. In *In: Caballero, B., Finglas, P.M., Toldrá, F. (Eds.), Encyclopedia of Food and Health* (Vol. 3, pp. 637–640). Elsevier - Academic Press. https://doi.org/10.1016/B978-0-12-384947-2.00441-4

Baech, S. B., Hansen, M., Bukhave, K., Jensen, M., Sørensen, S. S., Kristensen, L., Purslow, P. P., Skibsted, L. H., & Sandström, B. (2003). Nonheme-iron absorption from a phytate-rich meal is increased by the addition of small amounts of pork meat. *The American Journal of Clinical Nutrition*, *77*(1), 173–179. https://doi.org/https://doi.org/10.1093/ajcn/77.1.173

Baker, J. L., Michaelsen, K. F., & Rasmussen, K. M. (2004). Maternal prepregnant body mass index, duration of breastfeeding, and timing of complementary food introduction are associated with infant weight gain 1-3. *American Journal of Clinical Nutrition*, *80*(6), 1579–1588. https://doi.org/10.1093/ajcn/80.6.1579

Barański, M., Średnicka-Tober, D., Volakakis, N., Seal, C., Sanderson, R., Stewart, G. B., Benbrook, C., Biavati, B., Markellou, E., Giotis, C., Gromadzka-Ostrowska, J., Rembiałkowska, E., Skwarło-Sońta, K., Tahvonen, R., Janovská, D., Niggli, U., Nicot, P., & Leifert, C. (2014). Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analyses. *British Journal of Nutrition*, *112*, 794–811. https://doi.org/10.1017/S0007114514001366

Barnett, J. B., Hamer, D. H., & Meydani, S. N. (2010). Low zinc status: a new risk factor for pneumonia in the elderly? *Nutrition Reviews*, *68*(1), 30–37. https://doi.org/10.1111/j.1753-4887.2009.00253.x

Basulto, J., Manera, M., & Baladia, E. (2014). Ingesta dietética de nitratos en bebés y niños españoles y riesgo de metahemoglobinemia. *Pediatría Atención Primaria*, *16*(61), 65–69.

Beauchamp, G. K., & Mennella, J. A. (2011). Flavor perception in human infants: Development and functional significance. *Digestion*, *83*(SUPPL.1), 1–6. https://doi.org/10.1159/000323397

Bender, I., Ess, M., Matt, D., Moor, U., Tõnutare, T., & Luik, A. (2009). Quality of organic and conventional carrots. *Agronomy Research*, *7*(2), 572–577. https://www.researchgate.net/publication/268009822

Bernard-Bonnin, A.-C. (2006). Feeding problems of infants and toddlers. *Canadian Family Physician*, *52*, 1247–1251.

Bertinato, J. (2016). Copper: Physiology. In *Encyclopedia of Food and Health* (2nd ed., pp. 321–326). Elsevier – Academic Press. https://doi.org/10.1016/B978-0-12-384947-2.00201-4

Bjorklund, E. A., Heins, B. J., DiCostanzo, A., & Chester-Jones, H. (2014). Fatty acid profiles, meat quality, and sensory attributes of organic versus conventional dairy beef steers. *Journal of Dairy Science*, *97*(3), 1828–1834. https://doi.org/10.3168/jds.2013-6984

Boto Fidalgo, J. A. (2018). *LAS LEGUMBRES SECAS Alubias y garbanzos ANÁLISIS GLOBAL Experiencias de varios años*.

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International*, *43*(2), 414–431. https://doi.org/10.1016/j.foodres.2009.09.003

Braegger, C., Campoy, C., Colomb, V., Decsi, T., Domellof, M., Fewtrell, M., Hojsak, I., Mihatsch, W., Molgaard, C., Shamir, R., Turck, D., van Goudoever, J., & on Behalf of the ESPGHAN Committee on Nutrition. (2013). Vitamin D in the Healthy European Paediatric Population. *Journal of Pediatric Gastroenterology and Nutrition*, *56*(6), 692–701. https://doi.org/10.1097/MPG.0b013e31828f3c05

Brazinskiene, V., Asakaviciute, R., Miezeliene, A., Alencikiene, G., Ivanauskas, L., Jakstas, V., Viskelis, P., & Razukas, A. (2014). Effect of farming systems on the yield, quality parameters and sensory properties of conventionally and organically grown potato (Solanum tuberosum L.) tubers. *Food Chemistry*, *145*, 903–909. https://doi.org/10.1016/j.foodchem.2013.09.011

Brion, L. P., Bell, E. F., & Raghuveer, T. S. (2003). Vitamin E supplementation for prevention of morbidity and mortality in preterm infants. *Cochrane Database of Systematic Reviews*, *4*. https://doi.org/10.1002/14651858.cd003665

Brown, K. H., Peerson, J. M., Baker, S. K., & Hess, S. Y. (2009). Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. *Food and Nutrition Bulletin*, *30*(1 Suppl), S12–S40. https://doi.org/https://doi.org/10.1177/15648265090301S103

Butte, N., Cobb, K., Dwyer, J., Graney, L., Heird, W., & Rickard, K. (2004). The start healthy feeding guidelines for infants and toddlers. *Journal of the American Dietetic Association*, *104*(3), 442–454. https://doi.org/10.1016/j.jada.2004.01.027

Cámara, F., Barberá, R., Amaro, M. A., & Farré, R. (2007). Calcium, iron, zinc and copper transport and uptake by Caco-2 cells in school meals: Influence of protein and mineral interactions. *Food Chemistry*, *100*(3), 1085–1092. https://doi.org/10.1016/j.foodchem.2005.11.010

Cámara Martos, F., Marval León, J. R., & Moreno Rojas, R. (2015). Selenium bioavilability in foods: the role of speciation and interaction with other dietary components. In *W. Morrison (Ed.), Selenium: Dietary Sources, Properties and Role in Human Health* (1st ed., pp. 1–28). Nova Science Publishers.

Cámara-Martos, F., Ramírez-Ojeda, A. M., Jiménez-Mangas, M., Sevillano-Morales, J., & Moreno-Rojas, R. (2019). Selenium and cadmium in bioaccessible fraction of organic weaning food: Risk assessment and influence of dietary components. *Journal of Trace Elements in Medicine and Biology*, *56*, 116–123. https://doi.org/10.1016/j.jtemb.2019.08.004

Campbell, W. B., & López-Ortíz, S. (2014). *Sustainable Food Production Includes Human and Environmental Health | W. Bruce Campbell | Springer*. https://www.springer.com/gp/book/9789400774537

Campos Rivera, N. H., & Lagunes, I. R. (2014). Preferencias Alimentarias y su Asociación con Alimentos Saludables y No Saludables en Niños Preescolares. *Acta de Investigación Psicológica*, *4*(1), 1385–1397. https://doi.org/10.1016/s2007-4719(14)70382-5

Campos-Vega, R., Loarca-Piña, G., & Oomah, B. D. (2010). Minor components of pulses and their potential impact on human health. *Food Research International*, *43*(2), 461–482. https://doi.org/10.1016/j.foodres.2009.09.004

Carillo, P., Cacace, D., de Pascale, S., Rapacciuolo, M., & Fuggi, A. (2012). Organic vs. traditional potato powder. *Food Chemistry*, *133*(4), 1264–1273. https://doi.org/10.1016/j.foodchem.2011.08.088

Carvalho, L. M. J., Corrêa, M. M., Pereira, E. J., Nutti, M. R., Carvalho, J. L. V., Ribeiro, E. M. G., & Freitas, S. C. (2012). Iron and zinc retention in common beans (Phaseolus vulgaris L.) after home cooking. *Food and Nutrition Research*, *56*. https://doi.org/10.3402/fnr.v56i0.15618

Chung, M., Balk, E. M., Brendel, M., Ip, S., Lau, J., Lee, J., Lichtenstein, A., Patel, K., Raman, G., Tatsioni, A., Terasawa, T., & Trikalinos, T. A. (2009). Vitamin D and Calcium: A Systematic Review of Health Outcomes. *Evidence Report/Technology Assessment*, *183*, 1–140. www.ahrq.gov

Codex Alimentarius. (2007). *Organically produced foods*. World Health Organization.

Collins, J. F., Prohaska, J. R., & Knutson, M. D. (2010). Metabolic crossroads of iron and copper. *Nutrition Reviews*, *68*(3), 133–147. https://doi.org/10.1111/j.1753-4887.2010.00271.x

Comité de Nutrición. (2001). *GUIA DE ALIMENTACION PARA NIÑOS SANOS DE 0 A 2 AÑOS*.

Crecente-Campo, J., Nunes-Damaceno, M., Romero-Rodríguez, M. A., & Vázquez-Odériz, M. L. (2012). Color, anthocyanin pigment, ascorbic acid and total phenolic compound determination in organic versus conventional strawberries (Fragaria×ananassa Duch, cv Selva). *Journal of Food Composition and Analysis*, *28*(1), 23–30. https://doi.org/10.1016/j.jfca.2012.07.004

Cruz, O. M., Doren, V. A., Fernández, B. R., Salinas T, J. A., Urzúa B, S., & Tapia I, J. L. (2009). Intoxicación Neonatal por Sulfato de Magnesio: Caso clínico. *Revista Chilena de Pediatría*, *80*(3), 261–266.

Cuadros-Mendoza, C. A., Vichido-Luna, M. A., Montijo-Barrios, E., Zárate-Mondragón, F., Cadena-León, J. F., Cervantes-Bustamante, R., Toro-Monjáraz, E., & Ramírez-Mayans, J. A. (2017). Actualidades en alimentación complementaria. *Acta Pediátrica de México*, *38*(3), 182–201. https://doi.org/10.18233/apm38no3pp182-2011390

Cuypers, A., Plusquin, M., Remans, T., Jozefczak, M., Keunen, E., Gielen, H., Opdenakker, K., Nair, A. R., Munters, E., Artois, T. J., Nawrot, T., Vangronsveld, J., & Smeets, K. (2010). Cadmium stress: an oxidative challenge. *BioMetals*, *23*(5), 927–940. https://doi.org/10.1007/s10534-010-9329-x

Dahl, W. J., & Stewart, M. L. (2015). Position of the Academy of Nutrition and Dietetics: Health Implications of Dietary Fiber. *Journal of the Academy of Nutrition and Dietetics*, *115*(11), 1861–1870. https://doi.org/10.1016/j.jand.2015.09.003

de Benoist, B., Darnton-Hill, I., Davidsson, L., Fontaine, O., & Hotz, C. (2007). Conclusions of the Joint WHO/UNICEF/IAEA/IZiNCG Interagency Meeting on Zinc Status Indicators. *Food and Nutrition Bulletin*, *28*(3 Suppl), S480–S484. https://doi.org/https://doi.org/10.1177/15648265070283S306

de Ponti, T., Rijk, B., & van Ittersum, M. K. (2012). The crop yield gap between organic and conventional agriculture. *Agricultural Systems*, *108*, 1–9. https://doi.org/10.1016/j.agsy.2011.12.004

de Romaña, D. L., Olivares, M., Uauy, R., & Araya, M. (2011). Risks and benefits of copper in light of new insights of copper homeostasis. *Journal of Trace Elements in Medicine and Biology*, *25*(1), 3–13. https://doi.org/10.1016/j.jtemb.2010.11.004

de Souza Araújo, D. F., da Silva, A. M. R. B., de Andrade Lima, L. L., da Silva Vasconcelos, M. A., Andrade, S. A. C., & Asfora Sarubbo, L. (2014). The concentration of minerals and physicochemical contaminants in conventional and organic vegetables. *Food Control*, *44*, 242–248. https://doi.org/10.1016/j.foodcont.2014.04.005

Delaney, A. L., & Arvedson, J. C. (2008). Development of swallowing and feeding: Prenatal through first year of life. *Developmental Disabilities Research Reviews*, *14*, 105–117. https://doi.org/10.1002/ddrr.16

Dewey, K G, & Brown, K. H. (2003). Energy required from complementary foods and factors affecting intake of these foods. *Food and Nutrition Bulletin*, *24*(1), 5–28. https://doi.org/https://doi.org/10.1177/156482650302400102

Dewey, Kathryn G., & Adu-Afarwuah, S. (2008). Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries. *Maternal and Child Nutrition*, *4*, 24–85. https://doi.org/https://doi.org/10.1111/j.1740-8709.2007.00124.x

Dhuppar, P., Biyan, S. C., Chintapalli, B., & Sarveshwara Rao, D. (2012). Lentil Crop Production in the Context of Climate Change: An Appraisal. *Indian Research Journal of Extension Education, Special Issue*, *II*, 2012. http://faostat.fao.org

Domellöf, M., Szitanyi, P., Simchowitz, V., Franz, A., Mimouni, F., Braegger, C., Bronsky, J., Cai, W., Campoy, C., Carnielli, V., Darmaun, D., Decsi, T., Domellöf, M., Embleton, N., Fewtrell, M., Fidler Mis, N., Franz, A., Goulet, O., Hartman, C., … Yan, W. (2018). ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: Iron and trace minerals. *Clinical Nutrition*, *37*(6), 2354–2359. https://doi.org/10.1016/j.clnu.2018.06.949

Doyle, L. W., Crowther, C. A., Middleton, P., Marret, S., & Rouse, D. (2009). Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus (Review). In *Cochrane Database of Systematic Reviews* (Issue 1). https://doi.org/10.1002/14651858.CD004661.pub3

Duan, Y. S., & Shao, S. S. (2014). Inventory of china's pesticide in 2013 and prospects for 2014. *China Pet. Chem. Ind. Econ. Anal.*, *3*, 20–33.

Dwivedi, S. L., Sahrawat, K. L., Upadhyaya, H. D., Mengoni, A., Galardini, M., Bazzicalupo, M., Biondi, E. G., Hungria, M., Kaschuk, G., Blair, M. W., & Ortiz, R. (2015). Advances in Host Plant and Rhizobium Genomics to Enhance Symbiotic Nitrogen Fixation inGrain Legumes. *Advances in Agronomy*, *129*, 1–116. https://doi.org/10.1016/bs.agron.2014.09.001

EC. (2014). *Performance of agricultural policy | European Commission*. https://ec.europa.eu/info/food-farming-fisheries/farming/facts-and-figures/performance-agricultural-policy

EC. (2016). *Becoming an organic farmer | European Commission*. https://ec.europa.eu/info/food-farming-fisheries/farming/organic-farming/becoming-organic-farmer

EFSA. (2013). Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. *EFSA Journal*, *11*(10). https://doi.org/10.2903/j.efsa.2013.3408

EFSA. (2016). The 2014 European Union Report on Pesticide Residues in Food. *EFSA Journal*, *14*(10), 1–139. https://doi.org/10.2903/j.efsa.2016.4611

Egli, I. (2001). *Traditional food processing methods to increase mineral bioavailability from cereal and legume based weaning foods*. https://doi.org/10.3929/ethz-a-004132286

EUROSTAT. (2016). *Organic farming statistics*. https://ec.europa.eu/eurostat/statisticsexplained/

Everett, C. J., & Frithsen, I. L. (2008). Association of urinary cadmium and myocardial infarction. *Environmental Research*, *106*(2), 284–286. https://doi.org/10.1016/j.envres.2007.10.009

Fall, C. H. D., Borja, J. B., Osmond, C., Richter, L., Bhargava, S. K., Martorell, R., Stein, A. D., Barros, F. C., & Victora, C. G. (2011). Infant-feeding patterns and cardiovascular risk factors in young adulthood: Data from five cohorts in low- and middle-income countries. *International Journal of Epidemiology*, *40*(1), 47–62. https://doi.org/10.1093/ije/dyq155

FAO. (2000). *Food Safety and Quality as Affected by Organic Farming: Twenty Second FAO Regional Conference for Europe*. http://www.fao.org/3/X4983E/X4983E.htm

FAO. (2016a). *International Year of Pulses 2016*. http://www.fao.org/pulses-2016

FAO. (2016b). *Legumbres, semillas nutritivas para un futuro sostenible*.

FAOSTAT. (2018). *FAOSTAT*. http://www.fao.org/faostat/es/?#data/QC/visualize

Faria, M. A., Araújo, A., Pinto, E., Oliveira, C., Oliva-Teles, M. T., Almeida, A., Delerue-Matos, C., & Ferreira, I. M. P. L. V. O. (2018). Bioaccessibility and intestinal uptake of minerals from different types of home-cooked and ready-to-eat beans. *Journal of Functional Foods*, *50*, 201–209. https://doi.org/10.1016/j.jff.2018.10.001

Febles, C. I., Arias, A., Hardisson, A., Rodríguez-Álvarez, C., & Sierra, A. (2001). Phytic acid level in infant flours. *Food Chemistry*, *74*, 437–441. www.elsevier.com/locate/foodchem

FEN. (2017). *Informe sobre Legumbres, Nutrición y Salud*.

Fenner, K., Canonica, S., Wackett, L. P., & Elsner, M. (2013). Evaluating Pesticide Degradation in the Environment: Blind Spots and Emerging Opportunities. *Science*, *341*, 752–758. www.sciencemag.org

Feoktistova Victorava, L., & Clark Feoktistova, Y. (2018). El metabolismo del cobre. Sus consecuencias para la salud humana Metabolism of copper. Its consequences for human health. *Medisur*, *16*(4), 579–587.

FESNAD. (2010). *Ingestas dietéticas de referencia (IDR) para la población española*. https://www.researchgate.net/publication/202089075

Fewtrell, M., Bronsky, J., Campoy, C., Domellöf, M., Embleton, N., Mis, N. F., Hojsak, I., Hulst, J. M., Indrio, F., Lapillonne, A., & Molgaard, C. (2017). Complementary feeding: A position paper by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) committee on nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, *64*(1), 119–132. https://doi.org/10.1097/MPG.0000000000001454

Finch, C. W. (2015). Review of trace mineral requirements for preterm infants: What are the current recommendations for clinical practice? *Nutrition in Clinical Practice*, *30*(1), 44–58. https://doi.org/10.1177/0884533614563353

Finn, K., Jacquier, E., Kineman, B., Storm, H., & Carvalho, R. (2019). Nutrient intakes and sources of fiber among children with low and high dietary fiber intake: The 2016 feeding infants and toddlers study (FITS), a cross-sectional survey. *BMC Pediatrics*, *19*(1). https://doi.org/10.1186/s12887-019-1822-y

Fitsanakis, V. A., Zhang, N., Garcia, S., & Aschner, M. (2010). Manganese (Mn) and iron (Fe): Interdependency of transport and regulation. *Neurotoxicity Research*, *18*(2), 124–131. https://doi.org/10.1007/s12640-009-9130-1

Frisbie, S. H., Mitchell, E. J., Roudeau, S., Domart, F., Carmona, A., & Ortega, R. (2019). Manganese levels in infant formula and young child nutritional beverages in the United States and France: Comparison to breast milk and regulations. *PLoS ONE*, *14*(11). https://doi.org/10.1371/journal.pone.0223636

Frontela, C., Scarino, M. L., Ferruzza, S., Ros, G., & Martínez, C. (2009). Effect of dephytinization on bioavailability of iron, calcium and zinc from infant cereals assessed in the Caco-2 cell model. *World Journal of Gastroenterology*, *15*(16), 1977–1984. https://doi.org/10.3748/wjg.15.1977

Gaetke, L. M., Chow-Johnson, H. S., & Chow, C. K. (2014). Copper: toxicological relevance and mechanisms. *Archives of Toxicology*, *88*(11), 1929–1938. https://doi.org/10.1007/s00204-014-1355-y

Galiano Segovia, M. J., & Moreno Villares, J. M. (2016). Los alimentos orgánicos en la alimentación infantil Palabras clave. *Acta Pediátrica Española*, *74*(9), 225–230.

Gallagher, C. M., Chen, J. J., & Kovach, J. S. (2010). Environmental cadmium and breast cancer risk. *Aging*, *2*(11), 804–814. https://doi.org/https://doi.org/10.18632/aging.100226

Gąstoł, M., Domagała-Świątkiewicz, I., & Krośniak, M. (2011). Organic versus conventional–a comparative study on quality and nutritional value of fruit and vegetable juices. *Biological Agriculture and Horticulture*, *27*(3–4), 310–319. https://doi.org/10.1080/01448765.2011.648726

Gepts, P. (2001). Phaseolus vulgaris (Beans). In *Encyclopedia of Genetics* (pp. 1444–1445). Elsevier. https://doi.org/10.1006/rwgn.2001.1749

Gibson, R. S., & Hotz, C. (2000). The adequacy of micronutrients in complementary food. *Pediatrics*, *106*(Supplement 4), 1298–1300.

Gil Campos, M., Leis Trabazo, R., & Maldonado Lozano, J. (2017). Nutrición del niño de 1 a 3 años. In *Nutrición humana en el estado de salud* (Vol. 4, pp. 339–349).

Gil Hernández, A., Uauy Dagach, R., Dalmau Serra, J., Suárez Cortina, L., Aranceta Bartrina, J., Lama More, R. A., Martín Mateos, M., Martínez Suárez, V., & Pavón, P. (2006). Bases para una alimentación complementaria adecuada de los lactantes y los niños de corta edad. *Anales de Pediatria*, *65*(5), 481–495. https://doi.org/10.1157/13094263

Giusti, F., Capuano, E., Sagratini, G., & Pellegrini, N. (2019). A comprehensive investigation of the behaviour of phenolic compounds in legumes during domestic cooking and in vitro digestion. *Food Chemistry*, *285*, 458–467. https://doi.org/10.1016/j.foodchem.2019.01.148

Goldberger, J. R. (2011). Conventionalization, civic engagement, and the sustainability of organic agriculture. *Journal of Rural Studies*, *27*(3), 288–296. https://doi.org/10.1016/j.jrurstud.2011.03.002

Gomez-Pomar, E., Hatfield, E., Garlitz, K., Westgate, P. M., & Bada, H. S. (2018). Vitamin E in the Preterm Infant: A Forgotten Cause of Hemolytic Anemia. *American Journal of Perinatology*, *35*(3), 305–310. https://doi.org/10.1055/s-0037-1607283

Gomiero, T. (2018). Food quality assessment in organic vs. conventional agricultural produce: Findings and issues. *Applied Soil Ecology*, *123*, 714–728. https://doi.org/10.1016/j.apsoil.2017.10.014

González García, H. (2013). Metabolismo del hierro en niños. *Boletín de Pediatría*, *53*(226), 181–186.

González-Bernal, M. J., & Rubiales, D. (2016). Grain legumes in Spanish and European agriculture. *Arbor*, *192*(779). https://doi.org/10.3989/arbor.2016.779n3001

Gourama, H. (2015). A Preliminary Mycological Evaluation of Organic and Conventional Foods. *Food Protection Trends*, *35*(5), 385–391.

Grandjean, P., & Landrigan, P. J. (2014). Neurobehavioural effects of developmental toxicity. *The Lancet Neurology*, *13*(3), 330–338. https://doi.org/10.1016/S1474-4422(13)70278-3

Grantham-McGregor, S., & Baker-Henningham, H. (2010). Carencia de hierro en la infancia: Causas y consecuencias para el desarrollo infantil. *Annales Nestlé (Ed. Española)*, *68*(3), 107–120. https://doi.org/10.1159/000324426

Gregory, K. E., & Walker, W. A. (2013). Immunologic Factors in Human Milk and Disease Prevention in the Preterm Infant. *Current Pediatrics Reports*, *1*(4), 222–228. https://doi.org/10.1007/s40124-013-0028-2

Grummer-Strawn, L. M., & Mei, Z. (2004). Does Breastfeeding Protect Against Pediatric Overweight? Analysis of Longitudinal Data From the Centers for Disease Control and Prevention Pediatric Nutrition Surveillance System. *Pediatrics2*, *113*(2), e81–e86. https://doi.org/10.1542/peds.113.2.e81

Gruszfeld, D., & Socha, P. (2013). Early nutrition and health: Short-and long-term outcomes. *World Review of Nutrition and Dietetics*, *108*, 32–39. https://doi.org/10.1159/000351482

Guéguen, L., & Pascal, G. (2016). Organic Foods☆. In *Reference Module in Biomedical Sciences*. Elsevier. https://doi.org/10.1016/B978-0-12-801238-3.99501-1

Gutiérrez-Uribe, J. A., Guajardo-Flores, D., & López-Barrios, L. (2016). Legumes in the Diet. In *Encyclopedia of Food and Health* (pp. 539–543). Elsevier Inc. https://doi.org/10.1016/B978-0-12-384947-2.00420-7

Ha, J. H., Doguer, C., Wang, X., Flores, S. R., & Collins, J. F. (2016). High-iron consumption impairs growth and causes copper-deficiency anemia in weanling Sprague-Dawley rats. *PLoS ONE*, *11*(8). https://doi.org/10.1371/journal.pone.0161033

Halberg, N. (2012). Assessment of the environmental sustainability of organic farming: Definitions, indicators and the major challenges. *Canadian Journal of Plant Science*, *92*(6), 981–996. https://doi.org/10.4141/CJPS2012-035

Hall, C., Hillen, C., & Robinson, J. G. (2017). Composition, nutritional value, and health benefits of pulses. *Cereal Chemistry*, *94*(1), 11–31. https://doi.org/10.1094/CCHEM-03-16-0069-FI

Hallmann, E., Lipowski, J., Marszałek, K., & Rembiałkowska, E. (2013). The Seasonal Variation in Bioactive Compounds Content in Juice from Organic and Non-organic Tomatoes. *Plant Foods for Human Nutrition*, *68*(2), 171–176. https://doi.org/10.1007/s11130-013-0352-2

Hartwig, A. (2013). *Cadmium and Cancer* (pp. 491–507). https://doi.org/10.1007/978-94-007-5179-8_15

Haschke, F., Grathwohl, D., Detzel, P., Steenhout, P., Wagemans, N., & Erdmann, P. (2016). Postnatal High Protein Intake Can Contribute to Accelerated Weight Gain of Infants and Increased Obesity Risk. *Nestle Nutrition Institute Workshop Series*, *85*, 101–109. https://doi.org/10.1159/000439492

Hassan, A., Lassi, Z. S., & Bhutta, Z. A. (2012). Zinc supplementation for the promotion of growth and prevention of infections in infants less than six months of age. *Cochrane Database of Systematic Reviews*, *11*. https://doi.org/10.1002/14651858.cd010205

Herencia, J. F., García-Galavís, P. A., Dorado, J. A. R., & Maqueda, C. (2011). Comparison of nutritional quality of the crops grown in an organic and conventional fertilized soil. *Scientia Horticulturae*, *129*(4), 882–888. https://doi.org/10.1016/j.scienta.2011.04.008

Hicks, P. D., Hawthorne, K. M., Berseth, C. L., Marunycz, J. D., Heubi, J. E., & Abrams, S. A. (2012). Total calcium absorption is similar from infant formulas with and without prebiotics and exceeds that in human milk-fed infants. *BMC Pediatrics*, *12*(118). http://www.biomedcentral.com/1471-2431/12/118

Hill, H., & Lynchehaun, F. (2002). Organic milk: Attitudes and consumption patterns. *British Food Journal*, *104*(7), 526–542. https://doi.org/10.1108/00070700210434570

Honkanen, P., Verplanken, B., & Olsen, S. O. (2006). Ethical values and motives driving organic food choice. *Journal of Consumer Behaviour*, *5*(5), 420–430. https://doi.org/10.1002/cb.190

Hoogenboom, L. A. P., Bokhorst, J. G., Northolt, M. D., van de Vijver, L. P. L., Broex, N. J. G., Mevius, D. J., Meijs, J. A. C., & van der Roest, J. (2008). Contaminants and microorganisms in Dutch organic food products: A comparison with conventional products. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *25*(10), 1195–1207. https://doi.org/10.1080/02652030802014930

Howard, V. (2003). Organic food-the view of a medical toxico-pathologist. *British Journal of Midwifery*, *11*(5), 272–275.

Huber, M., Rembiałkowska, E., Średnicka, D., Bügel, S., & van de Vijver, L. P. L. (2011). Organic food and impact on human health: Assessing the status quo and prospects of research. *NJAS - Wageningen Journal of Life Sciences*, *58*(3–4), 103–109. https://doi.org/10.1016/j.njas.2011.01.004

Hughner, R. S., McDonagh, P., Prothero, A., Shultz, C. J., & Stanton, J. (2007). Who are organic food consumers? A compilation and review of why people purchase organic food. *Journal of Consumer Behaviour*, *6*(2–3), 94–110. https://doi.org/10.1002/cb.210

Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. *American Journal of Clinical Nutrition*, *91*(5), 1461S-1467S. https://doi.org/10.3945/ajcn.2010.28674F

Hurrell, R. F., Reddy, M. B., Juillerat, M.-A., & Cook, J. D. (2003). Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *American Journal of Clinical Nutrition*, *77*, 1213–1219. https://academic.oup.com/ajcn/article-abstract/77/5/1213/4689822

IFOAM. (2016). *The World of Organic Agriculture 2016.* FIBL & IFOAM - Organics International.

IFOAM. (2017). *IFOAM Standard | IFOAM*. https://www.ifoam.bio/our-work/how/standards-certification/organic-guarantee-system/ifoam-standard

Imdad, A., & Bhutta, Z. A. (2011). Effect of preventive zinc supplementation on linear growth in children under 5 years of age in developing countries: a meta-analysis of studies for input to the lives saved tool. *BMC Public Health*, *11*(Suppl 3). https://doi.org/10.1186/1471-2458-11-S3-S22

Iñiguez León, J. L. (2017). *Manejo de Alimentación Complementaria en los lactantes que asisten a Consulta Externa en el Hospital Universitario de Motupe en el periodo Diciembre 2015-Septiembre 2016*.

Institute of Medicine. (2005). *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. The National Academies Press. https://doi.org/https://doi.org/10.17226/10490.

Intawongse, M., & Dean, J. R. (2006). In-vitro testing for assessing oral bioaccessibility of trace metals in soil and food samples. *Trends in Analytical Chemistry*, *25*(9), 876–886. https://doi.org/10.1016/j.trac.2006.03.010

Järup, L., & Åkesson, A. (2009). Current status of cadmium as an environmental health problem. *Toxicology and Applied Pharmacology*, *238*(3), 201–208. https://doi.org/10.1016/j.taap.2009.04.020

Jodral-Segado, A. M., Navarro-Alarcón, M., López-Ga De La Serrana, H., & López-Martínez, M. C. (2003). Magnesium and calcium contents in foods from SE Spain: Influencing factors and estimation of daily dietary intakes. *Science of the Total Environment*, *312*(1–3), 47–58. https://doi.org/10.1016/S0048-9697(03)00199-2

Johansson, E., Hussain, A., Kuktaite, R., Andersson, S. C., & Olsson, M. E. (2014). Contribution of organically grown crops to human health. *International Journal of Environmental Research and Public Health*, *11*(4), 3870–3893. https://doi.org/10.3390/ijerph110403870

Joshi, M., Timilsena, Y., & Adhikari, B. (2017). Global production, processing and utilization of lentil: A review. *Journal of Integrative Agriculture*, *16*(12), 2898–2913. https://doi.org/10.1016/S2095-3119(17)61793-3

Joshi, V., Thatte, P., Prakash, J., & Jyothi Lakshmi, A. (2014). Effect of oilseed protein concentrates and exogenous amino acids on the dialysability of iron and zinc. *LWT - Food Science and Technology*, *59*(1), 540–546. https://doi.org/10.1016/j.lwt.2014.05.003

Jovani, M., Alegría, A., Barberá, R., Farré, R., Lagarda, M. J., & Clemente, G. (2000). Effect of proteins, phytates, ascorbic acid and citric acid on dialysability of calcium, iron, zinc and copper in soy-based infant formulas. *Nahrung*, *44*(2), 114–117. https://doi.org/https://doi.org/10.1002/(SICI)1521-3803(20000301)44:2<114::AID-FOOD114>3.0.CO;2-N

Kahl, J., Baars, T., Bügel, S., Busscher, N., Huber, M., Kusche, D., Rembiałkowska, E., Schmid, O., Seidel, K., Taupier-Letage, B., Velimirov, A., & Załęcka, A. (2012). Organic food quality: a framework for concept, definition and evaluation from the European perspective. *Journal of the Science of Food and Agriculture*, *92*(14), 2760–2765. https://doi.org/10.1002/jsfa.5640

Kalogeropoulos, N., Chiou, A., Ioannou, M., Karathanos, V. T., Hassapidou, M., & Andrikopoulos, N. K. (2010). Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries. *Food Chemistry*, *121*(3), 682–690. https://doi.org/10.1016/j.foodchem.2010.01.005

Kippler, M., Bakhtiar Hossain, M., Lindh, C., Moore, S. E., Kabir, I., Vahter, M., & Broberg, K. (2012). Early life low-level cadmium exposure is positively associated with increased oxidative stress. *Environmental Research*, *112*, 164–170. https://doi.org/10.1016/j.envres.2011.11.012

Knights, E., & Hobson, K. (2016). Chickpea: Overview. In *ENCYCLOPEDIA OF FOOD GRAINS* (Second, Vol. 1, pp. 316–323). Elsevier Ltd. https://doi.org/http://dx.doi.org/10.1016/B978-0-12-394437-5.00035-8

Kramer, M. S., & Kakuma, R. (2012). Optimal duration of exclusive breastfeeding. *Cochrane Database of Systematic Reviews*, *8*. https://doi.org/10.1002/14651858.CD003517.pub2

Kranz, S., Brauchla, M., Slavin, J. L., & Miller, K. B. (2012). What Do We Know about Dietary Fiber Intake in Children and Health? The Effects of Fiber Intake on Constipation, Obesity, and Diabetes in Children. *Advances in Nutrition*, *3*(1), 47–53. https://doi.org/10.3945/an.111.001362

Krebs, N. F., Miller, L. v., & Michael Hambidge, K. (2014). Zinc deficiency in infants and children: A review of its complex and synergistic interactions. *Paediatrics and International Child Health*, *34*(4), 279–288. https://doi.org/10.1179/2046905514Y.0000000151

Læssøe, J., Ljungdalh, A. K., Alrøe, H. F., Noe, E., Christensen, T., Dubgaard, A., Olsen, S. B., Kærgård, N., & Kastberg, P. (2014). Three perspectives on motivation and multicriteria assessment of organic food systems. *Ecology and Society*, *19*(3). https://doi.org/10.5751/ES-06397-190307

Lairon, D. (2010). Nutritional quality and safety of organic food. A review. *Agronomy for Sustainable Development*, *30*(1), 33–41. https://doi.org/10.1051/agro/2009019ï

Lazzerini, M. (2016). Oral zinc provision in acute diarrhea. *Current Opinion in Clinical Nutrition and Metabolic Care*, *19*(3), 239–243. https://doi.org/10.1097/MCO.0000000000000276

Leonhard, M. J., Chang, E. T., Loccisano, A. E., & Garry, M. R. (2019). A systematic literature review of epidemiologic studies of developmental manganese exposure and neurodevelopmental outcomes. *Toxicology*, *420*, 46–65. https://doi.org/10.1016/j.tox.2019.03.004

Leu, A. F. (2004). *Organic Agriculture Can Feed the World*.

Lev-Yadun, S., Gopher, A., & Abbo, S. (2000). The Cradle of Agriculture. *Science*, *288*, 1602–1603. https://doi.org/10.1126/science.288.5471.1602

Lewis, G. P., Schrire, B. D., Mackinder, B. A., Rico, L., & Clark, R. (2013). A 2013 linear sequence of legume genera set in a phylogenetic context - A tool for collections management and taxon sampling. *South African Journal of Botany*, *89*, 76–84. https://doi.org/10.1016/j.sajb.2013.06.005

Li, F. H., Guo, X. H., Xia, C. Y., Chen, L., Ling, B., & Ming, J. (2012). Research advance in antioxidant activity of phenolic compounds in whole grains. *Food Science*, *33*(13), 299–304.

Liebhardt, W. C. (2001). Get the facts straight: organic agriculture yields are good. *Organic Farming Research Foundation Information Bulletin*, 1–5. www.bioland.de

Lim, S., Ahn, S. Y., Song, I. C., Chung, M. H., Jang, H. C., Park, K. S., Lee, K. U., Pak, Y. K., & Lee, H. K. (2009). Chronic exposure to the herbicide, atrazine, causes mitochondrial dysfunction and insulin resistance. *PLoS ONE*, *4*(4). https://doi.org/10.1371/journal.pone.0005186

Liu, E., Pimpin, L., Shulkin, M., Kranz, S., Duggan, C. P., Mozaffarian, D., & Fawzi, W. W. (2018). Effect of zinc supplementation on growth outcomes in children under 5 years of age. *Nutrients*, *10*(3). https://doi.org/10.3390/nu10030377

Ljung, K., Palm, B., Grandér, M., & Vahter, M. (2011). High concentrations of essential and toxic elements in infant formula and infant foods - A matter of concern. *Food Chemistry*, *127*(3), 943–951. https://doi.org/10.1016/j.foodchem.2011.01.062

Llamas, F., & Acedo, C. (2016). Las leguminosas (Leguminosae o Fabaceae): una síntesis de las clasificaciones, taxonomía y filogenia de la familia a lo largo del tiempo. *AmboCiencias*, *14*, 5–18.

Lockie, S. (2006). Capturing the sustainability agenda: Organic foods and media discourses on food scares, environment, genetic engineering, and health. *Agriculture and Human Values*, *23*(3), 313–323. https://doi.org/10.1007/s10460-006-9007-3

Lombardo, S., Pandino, G., & Mauromicale, G. (2012). Nutritional and sensory characteristics of "early" potato cultivars under organic and conventional cultivation systems. *Food Chemistry*, *133*(4), 1249–1254. https://doi.org/10.1016/j.foodchem.2011.10.005

Lönnerdal, B., & Hernell, O. (2010). Homeostatic regulation of iron and its role in normal and abnormal iron status in infancy and childhood. *Annales Nestlé*, *68*(3), 96–104.

Lönnerdal, Bo. (2002). Phytic acid-trace element (Zn, Cu, Mn) interactions. *International Journal of Food Science and Technology*, *37*, 749–758.

Lönnerdal, Bo. (2005). Future Challenges of Nutrition in Pregnancy and Lactation. *Nestle Nutrition Workshop Series. Paediatric Programme*, *55*, 235–248.

López Amorós, M. L. (2000). *Estudio de compuestos fenólicos en legumbres. Influencia de la variedad y del proceso de germinación*. https://repositorio.uam.es/handle/10486/671954

López-Martínez, L. X., Leyva-López, N., Gutiérrez-Grijalva, E. P., & Heredia, J. B. (2017). Effect of cooking and germination on bioactive compounds in pulses and their health benefits. *Journal of Functional Foods*, *38*, 624–634. https://doi.org/10.1016/j.jff.2017.03.002

Los, F. G. B., Zielinski, A. A. F., Wojeicchowski, J. P., Nogueira, A., & Demiate, I. M. (2018). Beans (Phaseolus vulgaris L.): whole seeds with complex chemical composition. *Current Opinion in Food Science*, *19*, 63–71. https://doi.org/10.1016/j.cofs.2018.01.010

Luabeya, K. K. A., Mpontshane, N., Mackay, M., Ward, H., Elson, I., Chhagan, M., Tomkins, A., van den Broeck, J., & Bennish, M. L. (2007). Zinc or Multiple Micronutrient Supplementation to Reduce Diarrhea and Respiratory Disease in South African Children:

A Randomized Controlled Trial. *PLoS ONE*, *2*(6). https://doi.org/10.1371/journal.pone.0000541

Lucchini, R., Placidi, D., Cagna, G., Fedrighi, C., Oppini, M., Peli, M., & Zoni, S. (2017). Manganese and Developmental Neurotoxicity. In *Advances in Neurobiology* (Vol. 18, pp. 13–34). Springer New York LLC. https://doi.org/10.1007/978-3-319-60189-2_2

MacLean, W. C., van Dael, P., Clemens, R., Davies, J., Underwood, E., O'Risky, L., Rooney, D., & Schrijver, J. (2010). Upper levels of nutrients in infant formulas: Comparison of analytical data with the revised Codex infant formula standard. *Journal of Food Composition and Analysis*, *23*(1), 44–53. https://doi.org/10.1016/j.jfca.2009.07.008

Maldonado Lozano, J. (2018). La importancia de la nutrición en los primeros 1.000 días de la vida. *Acta Pediatr Esp*, *76*(3–4), e33-340.

Mamidi, S., Rossi, M., Annam, D., Moghaddam, S., Lee, R., Papa, R., & McClean, P. (2011). Investigation of the domestication of common bean (Phaseolus vulgaris) using multilocus sequence data. *Functional Plant Biology*, *38*(12), 953–967. https://doi.org/10.1071/FP11124

Marval-León, J. R., Cámara-Martos, F., Amaro-López, M. A., & Moreno-Rojas, R. (2014). Bioaccessibility and content of Se in fish and shellfish widely consumed in Mediterranean countries: Influence of proteins, fat and heavy metals. *International Journal of Food Sciences and Nutrition*, *65*(6), 678–685. https://doi.org/10.3109/09637486.2014.908173

Mazur, A., & Maier, J. A. M. (2016). Magnesium. In *Encyclopedia of Food and Health* (pp. 587–592). Elsevier Inc. https://doi.org/10.1016/B978-0-12-384947-2.00434-7

McCarty, M. F. (2012). Zinc and multi-mineral supplementation should mitigate the pathogenic impact of cadmium exposure. *Medical Hypotheses*, *79*(5), 642–648. https://doi.org/10.1016/j.mehy.2012.07.043

McCarty, M. F., & DiNicolantonio, J. J. (2014). Are organically grown foods safer and more healthful than conventionally grown foods? *British Journal of Nutrition*, *112*(10), 1589–1591. https://doi.org/10.1017/S0007114514002748

McDermid, J. M., & Lönnerdal, B. (2012). Iron. *Advances in Nutrition*, *3*(4), 532–533. https://doi.org/10.3945/an.112.002261

Megias Rangil, I., & Salas-Salvadó, J. (2019). Introducción a las dietas controladas en fibra. In *NUTRICIÓN Y DIETÉTICA CLÍNICA* (4ª, pp. 395–407). Elsevier.

Melø, R., Gellein, K., Evje, L., & Syversen, T. (2008). Minerals and trace elements in commercial infant food. *Food and Chemical Toxicology*, *46*(10), 3339–3342. https://doi.org/10.1016/j.fct.2008.08.007

Menéndez-Hernando, C., de La Huerga-López, A., Martín-Molina, R., Fariñas-Salto, M., & Chacón-Aguilar, R. (2019). Hipermagnesemia neonatal secundaria a intoxicación por laxante oral con sales de magnesio. *Revista Mexicana de Pediatría*, *86*(5), 194–196. https://doi.org/10.35366/sp195f

Mennella, J. A., Ventura, A. K., & Beauchamp, G. K. (2011). Differential growth patterns among healthy infants fed protein hydrolysate or cow-milk formulas. *Pediatrics*, *127*(1), 110–118. https://doi.org/10.1542/peds.2010-1675

Mercasa. (2017). *Alimentación en España 2017* (20ª).

MERCASA. (2019). *ALIMENTACIÓN EN ESPAÑA 2019*.

Mie, A., Andersen, H. R., Gunnarsson, S., Kahl, J., Kesse-Guyot, E., Rembiałkowska, E., Quaglio, G., & Grandjean, P. (2017). Human health implications of organic food and organic agriculture: a comprehensive review. *Environmental Health: A Global Access Science Source*, *16*(1). https://doi.org/10.1186/s12940-017-0315-4

Mir-Marqués, A., González-Masó, A., Cervera, M. L., & de La Guardia, M. (2015). Mineral profile of Spanish commercial baby food. *Food Chemistry*, *172*, 238–244. https://doi.org/10.1016/j.foodchem.2014.09.074

Mitchell, A. E., Hong, Y.-J., Koh, E., Barrett, D. M., Bryant, D. E., Denison, R. F., & Kaffka, S. (2007). Ten-Year Comparison of the Influence of Organic and Conventional Crop Management Practices on the Content of Flavonoids in Tomatoes. *Journal of Agricultural and Food Chemistry*, *55*(15), 6154–6159. https://doi.org/10.1021/jf070344

Mnif, W., Hassine, A. I. H., Bouaziz, A., Bartegi, A., Thomas, O., & Roig, B. (2011). Effect of endocrine disruptor pesticides: A review. *International Journal of Environmental Research and Public Health*, *8*(6), 2265–2303. https://doi.org/10.3390/ijerph8062265

Moráis López, A., Dalmau Serra, J., & Comité de Nutrición de la AEP. (2011). Iron deficiency in infants and toddlers: Impact on health and preventive strategies. *Anales de Pediatría*, *74*(6), 415.e1-415.e10. https://doi.org/10.1016/j.anpedi.2011.01.036

Moreda-Piñeiro, J., Moreda-Piñeiro, A., Romarís-Hortas, V., Domínguez-González, R., Alonso-Rodríguez, E., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D., & Bermejo-Barrera, P. (2013). ICP-MS for the determination of selenium bioavailability from seafood and effect of major food constituents. *Microchemical Journal*, *108*, 174–179. https://doi.org/10.1016/j.microc.2012.10.019

Müller, T., & Tanner, S. (2017). Disorders of Copper Metabolism. In *Diseases of the Liver and Biliary System in Children* (Fourth, pp. 323–339).

Mulvihill, B., & Morrissey, P. A. (1998). Influence of the sulphydryl content of animal proteins on in vitro bioavailability of non-haem iron. *Food Chemistry*, *61*(1/2), 1–7. https://doi.org/10.1016/s0308-8146(97)00127-1

Nagata, C., Nagao, Y., Nakamura, K., Wada, K., Tamai, Y., Tsuji, M., Yamamoto, S., & Kashiki, Y. (2013). Cadmium exposure and the risk of breast cancer in Japanese women. *Breast*

Cancer Research and Treatment, *138*(1), 235–239. https://doi.org/10.1007/s10549-013-2414-4

Naozuka, J., & Oliveira, P. v. (2012). Cooking Effects on Iron and Proteins Content of Beans (Phaseolus Vulgaris L.) by GF AAS and MALDI-TOF MS. *Journal of the Brazilian Chemical Society*, *23*(1), 156–162.

Nawrot, T. S., Staessen, J. A., Roels, H. A., Munters, E., Cuypers, A., Richart, T., Ruttens, A., Smeets, K., Clijsters, H., & Vangronsveld, J. (2010). Cadmium exposure in the population: From health risks to strategies of prevention. *BioMetals*, *23*(5), 769–782. https://doi.org/10.1007/s10534-010-9343-z

Neal, A. P., & Guilarte, T. R. (2013). Mechanisms of lead and manganese neurotoxicity. *Toxicology Research*, *2*(2), 99–114. https://doi.org/10.1039/c2tx20064c

Niggli, U. (2014). Sustainability of organic food production: Challenges and innovations. *Proceedings of the Nutrition Society*, *74*(1), 83–88. https://doi.org/10.1017/S0029665114001438

Olausson, H., Goldberg, G. R., Ann Laskey, M., Schoenmakers, I., Jarjou, L. M. A., & Prentice, A. (2012). Calcium economy in human pregnancy and lactation. *Nutrition Research Reviews*, *25*(1), 40–67. https://doi.org/10.1017/S0954422411000187

Olivares, M., Pizarro, F., de Romaña, D. L., & Ruz, M. (2010). Acute copper supplementation does not inhibit non-heme iron bioavailability in humans. *Biological Trace Element Research*, *136*(2), 180–186. https://doi.org/10.1007/s12011-009-8536-1

Oliveira, A. B., Moura, C. F. H., Gomes-Filho, E., Marco, C. A., Urban, L., & Miranda, M. R. A. (2013). The Impact of Organic Farming on Quality of Tomatoes Is Associated to Increased Oxidative Stress during Fruit Development. *PLoS ONE*, *8*(2). https://doi.org/10.1371/journal.pone.0056354

Olmedilla Alonso, B., Farré Rovir, R., Asensio Vegas, C., & Martín Pedrosa, M. (2010). Papel de las leguminosas en la alimentación actual. *Actividad Dietetica*, *14*(2), 72–76. https://doi.org/10.1016/S1138-0322(10)70014-6

OMS. (2011). *Directriz: Administración de suplementos de vitamina A lactantes y niños 6–59 meses de edad.* www.who.int

OMS. (2013). *Nutrición del lactante*. https://www.who.int/topics/infant_nutrition/es/

OMS. (2015). *Alimentación complementaria*. https://www.who.int/elena/titles/complementary_feeding/es/

OPS/OMS. (2010). *La alimentación del lactante y del niño pequeño Capítulo Modelo para libros de texto dirigidos a estudiantes de medicina y otras ciencias de la salud*.

PAHO/WHO. (2003). *GUIDING PRINCIPLES FOR COMPLEMENTARY FEEDING OF THE BREASTFEED CHILD*.

PAN. (2009). *The List of Lists. A catalogue of lists of pesticides identifying those associated with particularly harmful or environmental impacts*. http://unece.org/env/lrtap/

Pandelova, M., Lopez, W. L., Michalke, B., & Schramm, K. W. (2012). Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: Comparison of assessed infant intakes with the present safety limits for minerals and trace elements. *Journal of Food Composition and Analysis*, *27*(2), 120–127. https://doi.org/10.1016/j.jfca.2012.04.011

Pappas, A. C., Zoidis, E., Georgiou, C. A., Demiris, N., Surai, P. F., & Fegeros, K. (2011). Influence of organic selenium supplementation on the accumulation of toxic and essential trace elements involved in the antioxidant system of chicken. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *28*(4), 446–454. https://doi.org/10.1080/19440049.2010.549152

Pardío-López, L. M. J. (2012). Alimentación complementaria del niño de seis a 12 meses de edad. *Acta Pediátrica de México*, *33*(2), 80–88. www.nietoeditores.com.mx

Pechin, G. H. (2012). Absorción, metabolismo y homeostasis del zinc en los animales y el hombre. *Ciencia Veterinaria*, *14*(1), 93–125.

Pehrsson, P. R., Patterson, K. Y., & Khan, M. A. (2014). Selected vitamins, minerals and fatty acids in infant formulas in the United States. *Journal of Food Composition and Analysis*, *36*(1–2), 66–71. https://doi.org/10.1016/j.jfca.2014.06.004

Pérez González, E., Pérez González, E., Santos Rodríguez, F., & Coto García, E. (2009). *Homeostasis del magnesio. Etiopatogenia, clínica y tratamiento de la hipomagnesemia. A propósito de un caso*. http://www.senefro.org

Pérez-Llamas, F., Marín, J. F., Larqué, E., Garaulet, M., & Zamora, S. (2003). Effect of protein hydrolysis on the dialysability of amino acids and minerals in infant formulas. *Journal of Physiology and Biochemistry*, *59*(1), 19–24.

Pieper, J. R., & Barrett, D. M. (2009). Effects of organic and conventional production systems on quality and nutritional parameters of processing tomatoes. *Journal of the Science of Food and Agriculture*, *89*(2), 177–194. https://doi.org/10.1002/jsfa.3437

Pimentel, D. (2010). The effects of antibiotic and pesticide resistance on public health. In: IOM (Institute of Medicine). In *Antibiotic Resistance: Implications for Global Health and Novel Intervention Strategies: Workshop Summary*. The National Academies Press. https://doi.org/10.17226/12925

Polanska, K., Krol, A., Sobala, W., Gromadzinska, J., Brodzka, R., Calamandrei, G., Chiarotti, F., Wasowicz, W., & Hanke, W. (2016). Selenium status during pregnancy and child psychomotor development - Polish Mother and Child Cohort study. *Pediatric Research*, *79*(6), 863–869. https://doi.org/10.1038/pr.2016.32

Popa, M. E., Mitelut, A. C., Popa, E. E., Stan, A., & Popa, V. I. (2019). Organic foods contribution to nutritional quality and value. *Trends in Food Science and Technology*, *84*, 15–18. https://doi.org/10.1016/j.tifs.2018.01.003

Pretty, J., & Hine, R. (2001). *Reducing Food Poverty with Sustainable Agriculture: A Summary of New Evidence Final Report from the "SAFE-World" (The Potential of Sustainable Agriculture to Feed the World) Research Project*.

Rachwa-Rosiak, D., Nebesny, E., & Budryn, G. (2015). Chickpeas—composition, nutritional Value, health Benefits, application to bread and snacks: A Review. *Critical Reviews in Food Science and Nutrition*, *55*(8), 1137–1145. https://doi.org/10.1080/10408398.2012.687418

Ramírez-Cárdenas, L., Leonel, A. J., & Brunoro Costa, N. M. (2008). Efeito do processamento doméstico sobre o teor de nutrientes e de fatores antinutricionais de diferentes cultivares de feijão comum. *Ciência e Tecnologia de Alimentos*, *28*(1), 200–213. https://doi.org/10.1590/S0101-20612008000100029

Ramírez-Ojeda, A. M., Moreno-Rojas, R., & Cámara-Martos, F. (2018). Mineral and trace element content in legumes (lentils, chickpeas and beans): Bioaccesibility and probabilistic assessment of the dietary intake. *Journal of Food Composition and Analysis*, *73*, 17–28. https://doi.org/10.1016/j.jfca.2018.07.007

Rasane, P., Jha, A., Kumar, A., & Sharma, N. (2014). Reduction in phytic acid content and enhancement of antioxidant properties of nutricereals by processing for developing a fermented baby food. *Journal of Food Science and Technology*, *52*(6), 3219–3234. https://doi.org/10.1007/s13197-014-1375-x

Reganold, J. P., Glover, J. D., Andrews, P. K., & Hinman, H. R. (2001). Sustainability of three apple production systems. *Nature*, *410*, 926–930.

Rembiakowska, E. (2016). Organic Food: Effect on Nutrient Composition. In *Encyclopedia of Food and Health* (pp. 171–177). Elsevier Inc. https://doi.org/10.1016/B978-0-12-384947-2.00505-5

Rigo, J., Pieltain, C., Christmann, V., Bonsante, F., Moltu, S. J., Iacobelli, S., & Marret, S. (2017). Serum Magnesium Levels in Preterm Infants Are Higher Than Adult Levels: A Systematic Literature Review and Meta-analysis. *Nutrients*, *9*(10). https://doi.org/10.3390/nu9101125

Roberts, J. R., Karr, C. J., & Council On Environmental Health. (2012). Pesticide Exposure in Children. *PEDIATRICS*, *130*(6), e1765–e1788. https://doi.org/10.1542/peds.2012-2758

Rodríguez, E. M., Sanz Alaejos, M., & Díaz Romero, C. (2002). Concentraciones de calcio, magnesio, sodio y potasio en leche materna y fórmulas de inicio. *Archivos Latinoamericanos de Nutrición*, *52*(4), 406–412. http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0004-06222002000400013&lng=es&nrm=iso&tlng=es

Rodríguez-Barranco, M., Lacasaña, M., Aguilar-Garduño, C., Alguacil, J., Gil, F., González-Alzaga, B., & Rojas-García, A. (2013). Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: A systematic review and meta-analysis. *Science of the Total Environment*, *454–455*, 562–577. https://doi.org/10.1016/j.scitotenv.2013.03.047

Roels, H. A., Bowler, R. M., Kim, Y., Claus Henn, B., Mergler, D., Hoet, P., Gocheva, V. v., Bellinger, D. C., Wright, R. O., Harris, M. G., Chang, Y., Bouchard, M. F., Riojas-Rodriguez, H., Menezes-Filho, J. A., & Téllez-Rojo, M. M. (2012). Manganese exposure and cognitive deficits: A growing concern for manganese neurotoxicity. *NeuroToxicology*, *33*(4), 872–880. https://doi.org/10.1016/j.neuro.2012.03.009

Romero-Velarde, E., Villalpando-Carrión, S., Pérez-Lizaur, A. B., Iracheta-Gerez, M. de la L., Alonso-Rivera, C. G., López-Navarrete, G. E., García-Contreras, A., Ochoa-Ortiz, E., Zarate-Mondragón, F., López-Pérez, G. T., Chávez-Palencia, C., Guajardo-Jáquez, M., Vázquez-Ortiz, S., Pinzón-Navarro, B. A., Torres-Duarte, K. N., Vidal-Guzmán, J. D., Michel-Gómez, P. L., López-Contreras, I. N., Arroyo-Cruz, L. V., … Pinacho-Velázquez, J. L. (2016). Consenso para las prácticas de alimentación complementaria en lactantes sanos. *Boletin Medico Del Hospital Infantil de Mexico*, *73*(5), 338–356. https://doi.org/10.1016/j.bmhimx.2016.06.007

Ros, L., & Ros, I. (2010). Cobre y cinc en pediatría. *Anales de Pediatria Continuada*, *8*(4), 191–195. https://doi.org/10.1016/S1696-2818(10)70034-2

Ruiz de Maya, S., López-López, I., & Munuera, J. L. (2011). Organic food consumption in Europe: International segmentation based on value system differences. *Ecological Economics*, *70*(10), 1767–1775. https://doi.org/10.1016/j.ecolecon.2011.04.019

Sandoval Jurado, L., Jiménez Báez, M. V., Olivares Juárez, S., & de la Cruz Olvera, T. (2016). Lactancia materna, alimentación complementaria y el riesgo de obesidad infantil. *Atencion Primaria*, *48*(9), 572–578. https://doi.org/10.1016/j.aprim.2015.10.004

Sarmento, T. R. (2012). *Impacto del procesamiento sobre la pared celular y las propiedades hipoglucémicas y tecnofuncionales de leguminosas*.

Schipanski, M. E., & Drinkwater, L. E. (2012). Nitrogen fixation in annual and perennial legume-grass mixtures across a fertility gradient. *Plant and Soil*, *357*(1), 147–159. https://doi.org/10.1007/s11104-012-1137-3

SEGHNP-AEP. (2010). *Protocolos diagnóstico-terapéuticos de gastroenterología, hepatología y nutrición pediátrica SEGHNP-AEP*. Ergon.

Serefko, A., Szopa, A., & Poleszak, E. (2016). Magnesium and depression. *Magnesium Research*, *29*(3), 112–119. https://doi.org/10.1684/mrh.2016.0407

Seufert, V., Ramankutty, N., & Foley, J. A. (2012). Comparing the yields of organic and conventional agriculture. *Nature*, *485*(7397), 229–232. https://doi.org/10.1038/nature11069

Sharma, N., & Singhvi, R. (2018). Consumers perception and Behaviour towards organic food: A systematic review of literature. *Journal of Pharmacognosy and Phytochemistry*, *7*(2), 2152–2155. http://www.codexalimentarius.org/standards/thema

Sharp, P. (2004). The molecular basis of copper and iron interactions. *Proceedings of the Nutrition Society*, *63*(4), 563–569. https://doi.org/10.1079/pns2004386

Sheng, J.-P., Liu, C., & Shen, L. (2009). Guang pu xue yu guang pu fen xi = Guang pu. *Spectroscopy and Spectral Analysis*, *29*(8), 2244–2246.

Skröder, H. M., Hamadani, J. D., Tofail, F., Persson, L. Å., Vahter, M. E., & Kippler, M. J. (2015). Selenium status in pregnancy influences children's cognitive function at 1.5 years of age. *Clinical Nutrition*, *34*(5), 923–930. https://doi.org/10.1016/j.clnu.2014.09.020

Smith-Spangler, C., Brandeau, M. L., Hunter, G. E., Clay Bavinger, J., Pearson, M., Eschbach, P. J., Sundaram, V., Liu, H., Schirmer, P., Stave, C., Olkin, I., & Bravata, D. M. (2012). Are Organic Foods Safer or Healthier Than Conventional Alternatives? A Systematic Review. *Annals of Internal Medicine*, *157*(5), 348–366. https://doi.org/https://doi.org/10.7326/0003-4819-157-5-201209040-00007

Solomons, N. W. (2008). Iron Deficiency and Other Nutrient Deficiencies. *Pediatric Nutrition in Practice*, 137–141.

Souad, C., Farida, Z., Nadra, L., François, B., Bougle, D., & Azeddine, S. (2006). Trace element level in infant hair and diet, and in the local environment of the Moroccan city of Marrakech. *Science of the Total Environment*, *370*(2–3), 337–342. https://doi.org/10.1016/j.scitotenv.2006.06.020

Strumylaite, L., Kregzdyte, R., Bogusevicius, A., Poskiene, L., Baranauskiene, D., & Pranys, D. (2014). Association between cadmium and breast cancer risk according to estrogen receptor and human epidermal growth factor receptor 2: Epidemiological evidence. *Breast Cancer Research and Treatment*, *145*(1), 225–232. https://doi.org/10.1007/s10549-014-2918-6

Sughis, M., Penders, J., Haufroid, V., Nemery, B., & Nawrot, T. S. (2011). Bone resorption and environmental exposure to cadmium in children: a cross-sectional study. *Environmental Health*, *10*. https://doi.org/https://doi.org/10.1186/1476-069X-10-104

Talhaoui, N., Taamalli, A., Gómez-Caravaca, A. M., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2015). Phenolic compounds in olive leaves: Analytical determination, biotic and abiotic influence, and health benefits. *Food Research International*, *77*, 92–108. https://doi.org/10.1016/j.foodres.2015.09.011

Tellez-Plaza, M., Guallar, E., Howard, B. v., Umans, J. G., Francesconi, K. A., Goessler, W., Silbergeld, E. K., Devereux, R. B., & Navas-Acien, A. (2013). Cadmium exposure and incident cardiovascular disease. *Epidemiology*, *24*(3), 421–429. https://doi.org/10.1097/EDE.0b013e31828b0631

Tétard-Jones, C., Shotton, P. N., Rempelos, L., Cooper, J., Eyre, M., Orr, C. H., Leifert, C., & Gatehouse, A. M. R. (2013). Quantitative proteomics to study the response of wheat to contrasting fertilisation regimes. *Molecular Breeding*, *31*(2), 379–393. https://doi.org/10.1007/s11032-012-9796-6

Teucher, B., Olivares, M., & Cori, H. (2004). Enhancers of iron absorption: Ascorbic acid and other organic acids. *International Journal for Vitamin and Nutrition Research*, *74*(6), 403–419. https://doi.org/10.1024/0300-9831.74.6.403

Tong, G. M., & Rude, R. K. (2005). Magnesium deficiency in critical illness. *Journal of Intensive Care Medicine*, *20*(1), 3–17. https://doi.org/10.1177/0885066604271539

Tosh, S. M., & Yada, S. (2010). Dietary fibres in pulse seeds and fractions: Characterization, functional attributes, and applications. *Food Research International*, *43*(2), 450–460. https://doi.org/10.1016/j.foodres.2009.09.005

Traber, M. G., & Stevens, J. F. (2011). Vitamins C and E: Beneficial effects from a mechanistic perspective. *Free Radical Biology and Medicine*, *51*(5), 1000–1013. https://doi.org/10.1016/j.freeradbiomed.2011.05.017

Tripathi, R. M., Raghunath, R., Mahapatra, S., & Sadasivan, S. (2001). Blood lead and its effect on Cd, Cu, Zn, Fe and hemoglobin levels of children. *The Science of the Total Environment*, *277*(1–3), 161–168. https://doi.org/https://doi.org/10.1016/s0048-9697(00)00871-8

USDA. (2016). *Organic Regulations | Agricultural Marketing Service*. https://www.ams.usda.gov/rules-regulations/organic

Vallverdú-Queralt, A., Medina-Remón, A., Casals-Ribes, I., & Lamuela-Raventos, R. M. (2012). Is there any difference between the phenolic content of organic and conventional tomato juices? *Food Chemistry*, *130*(1), 222–227. https://doi.org/10.1016/j.foodchem.2011.07.017

van Dijk, M., Hunnius, S., & van Geert, P. (2009). Variability in eating behavior throughout the weaning period. *Appetite*, *52*(3), 766–770. https://doi.org/10.1016/j.appet.2009.02.001

van Maele-Fabry, G., Hoet, P., Vilain, F., & Lison, D. (2012). Occupational exposure to pesticides and Parkinson's disease: A systematic review and meta-analysis of cohort studies. *Environment International*, *46*, 30–43. https://doi.org/10.1016/j.envint.2012.05.004

van Wyk, B. E., & Boatwright, J. S. (2013). Advances in legume systematics 12. *South African Journal of Botany*, *89*, 1–2. https://doi.org/10.1016/j.sajb.2013.08.001

Vasishtha, H., & Srivastava, R. P. (2013). Effect of soaking and cooking on dietary fibre components of different type of chickpea genotypes. *Journal of Food Science and Technology*, *50*(3), 579–584. https://doi.org/10.1007/s13197-011-0366-4

Vásquez Garibay, E. M., Romero Velarde, E. R., & Larrosa Haro, A. (2012). Recomendaciones para la alimentación del niño durante los primeros 23 meses de vida. *Pediatría de México*, *14*(1), 25–42. www.medigraphic.com/pediatriademexico

Velasco-Reynold, C., Navarro-Alarcon, M., Lopez Ga De La Serrana, H., Perez-Valero, V., Agil, A., & Lopez-Martinez, M. C. (2010). Dialysability of magnesium and calcium from hospital duplicate meals: Influence exerted by other elements. *Biological Trace Element Research*, *133*(3), 313–324. https://doi.org/10.1007/s12011-009-8444-4

Velasco-Ryenold, C., Navarro-Alarcón, M., Lopez-Ga De La Serrana, H., Perez-Valero, V., & Lopez-Martinez, M. C. (2008). Total and dialyzable levels of manganese from duplicate meals and influence of other nutrients: Estimation of daily dietary intake. *Food Chemistry*, *109*(1), 113–121. https://doi.org/10.1016/j.foodchem.2007.12.025

Ventura, A. K., & Worobey, J. (2013). Early influences on the development of food preferences. *Current Biology*, *23*(9), R401–R403. https://doi.org/10.1016/j.cub.2013.02.037

Vinha, A. F., Barreira, S. V. P., Costa, A. S. G., Alves, R. C., & Oliveira, M. B. P. P. (2014). Organic versus conventional tomatoes: Influence on physicochemical parameters, bioactive compounds and sensorial attributes. *Food and Chemical Toxicology*, *67*, 139–144. https://doi.org/10.1016/j.fct.2014.02.018

Vioque, J., Sánchez-Vioque, R., Pedroche, J., Yust, M. del M., & Millán, F. (2001). Obtención y aplicaciones de concentrados y aislados protéicos. *Grasas y Aceites*, *52*(2), 127–131. https://www.researchgate.net/publication/26523743

Vrček, I. V., Čepo, D. V., Rašić, D., Peraica, M., Žuntar, I., Bojić, M., Mendaš, G., & Medić-Šarić, M. (2014). A comparison of the nutritional value and food safety of organically and conventionally produced wheat flours. *Food Chemistry*, *143*, 522–529. https://doi.org/10.1016/j.foodchem.2013.08.022

WHO. (2001). *THE OPTIMAL DURATION OF EXCLUSIVE BREASTFEEDING REPORT OF AN EXPERT CONSULTATION*. http://www.who.int/child-adolescent-health

WHO. (2005). *Guiding principles for feeding non-breastfed children 6-24 months of age*.

WHO. (2008). *Strengthening action to improve feeding of infants and young children 6-23 months of age in nutrition and child health programmes*.

WHO/UNICEF. (2003). *Global strategy for infant and young child feeding*. World Health Organization.

WHO/UNICEF. (2004). *CLINICAL MANAGEMENT OF ACUTE DIARRHOEA*.

Willoughby, J. L., & Bowen, C. N. (2014). Zinc deficiency and toxicity in pediatric practice. *Current Opinion in Pediatrics*, *26*(5), 579–584. https://doi.org/10.1097/MOP.0000000000000132

Winter, C. K., & Davis, S. F. (2006). Organic Foods. *Journal of Food Science*, *71*(9), R117–R124. https://doi.org/10.1111/j.1750-3841.2006.00196.x

Wu, P., Qin, Z. W., Zhou, X. Y., Wu, T., & Xin, M. (2011). Research progress of pesticide residues in vegetables. *Journal of Northeast Agricultural University*, *42*(1), 138–144. https://www.cabdirect.org/cabdirect/abstract/20113342777

Xu, H. L., Wang, R., Xu, R. Y., Mridha, M. A. U., & Goyal, S. (2003). Yield and quality of leafy vegetables grown with organic fertilizations. *Acta Horticulturae*, *627*, 25–33. https://doi.org/10.17660/ActaHortic.2003.627.2

Yu, X., Guo, L., Jiang, G., Song, Y., & Muminov, M. A. (2018). Advances of organic products over conventional productions with respect to nutritional quality and food security. *Acta Ecologica Sinica*, *38*(1), 53–60. https://doi.org/10.1016/j.chnaes.2018.01.009

Zander, K., & Hamm, U. (2010). Consumer preferences for additional ethical attributes of organic food. *Food Quality and Preference*, *21*(5), 495–503. https://doi.org/10.1016/j.foodqual.2010.01.006

Zhang, Z. B., Jiao, Z. P., Li, H. Y., & Lei, H. (2014). Comparison of mineral elements ion organic and traditional black sesame. *Chemical and Biomolecular Engineering*, *31*(5), 72–74.

Zulet, M. A., & Martínez, J. A. (2001). Dieta mediterránea: legumbres y colesterolemia. *Revista Chilena de Nutrición*, *28*, 312–320.

Objetivos

2. OBJETIVOS

En este contexto, esta Tesis Doctoral tiene como objetivo proporcionar información acerca de la biodisponibilidad de elementos inorgánicos de leguminosas y potitos ecológicos. Se plantean los siguientes objetivos:

Objetivo 1: Estudiar el contenido total de elementos inorgánicos (Ca, Mg, Fe, Zn, Cu y Mn) presentes en tres variedades de leguminosas principalmente consumidas en España (judías, garbanzos y lentejas) y en los dos tipos de formatos en los que se comercializan (secas o ya cocidas, listas para su consumo). Analizar las contribuciones a las Ingestas Dietéticas de Referencia (IDR) de estos micronutrientes para la población española a partir del consumo de estas leguminosas (Capítulo 2).

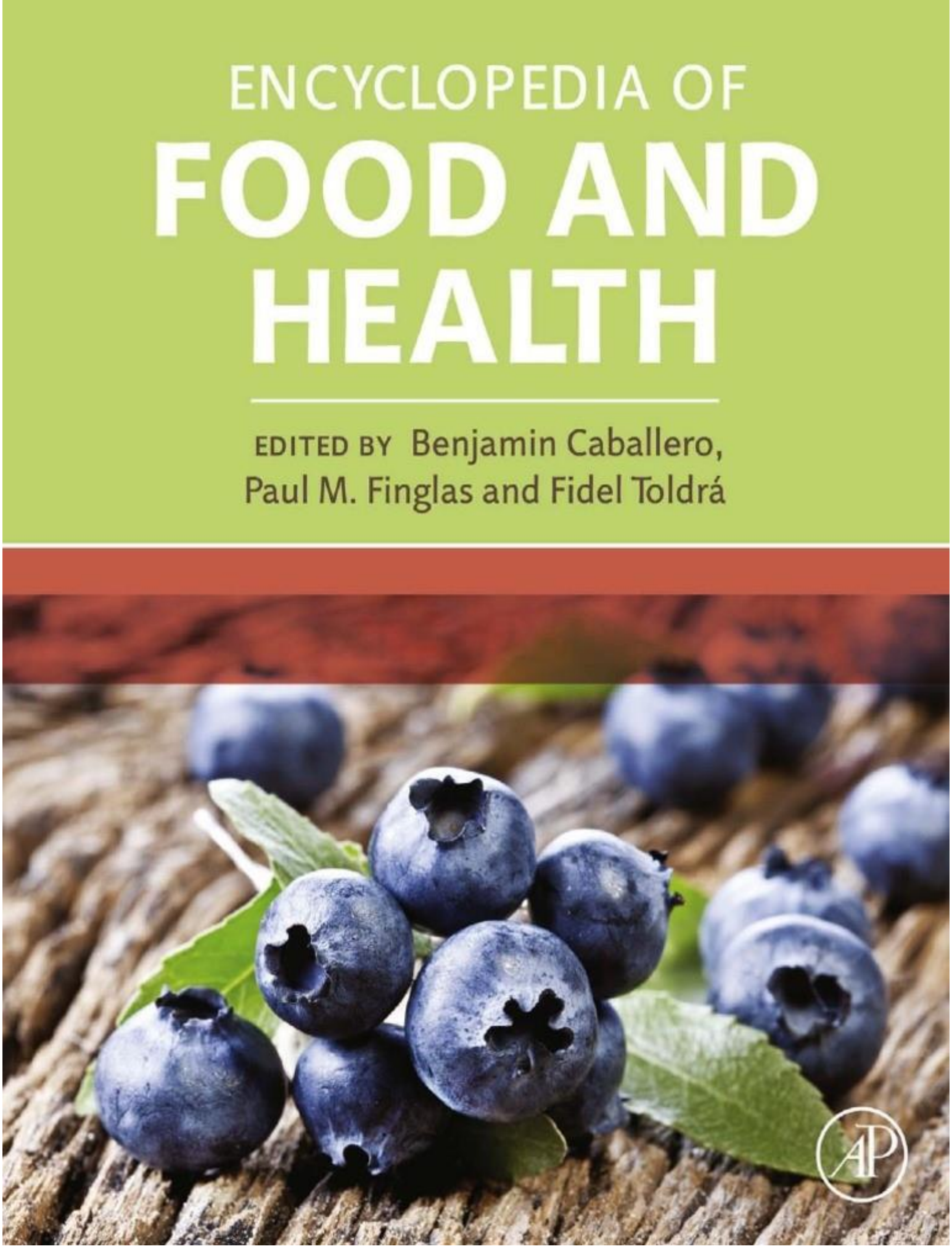
Objetivo 2: Analizar la bioaccesibilidad de elementos inorgánicos (Ca, Mg, Fe, Zn, Cu y Mn) presentes en las tres variedades de leguminosas (judías, garbanzos y lentejas), su contenido en otros principios inmediatos (proteínas y grasa) y la influencia de éstos últimos en los valores de bioaccesibilidad de elementos inorgánicos obtenidos. Estudiar la influencia del procesado en la calidad nutricional de este grupo de alimentos (Capítulo 2).

Objetivo 3: Estudiar el contenido total de elementos inorgánicos (Ca, Mg, Fe, Zn, Cu, Mn, Se y Cd) presentes en una serie de formulaciones de alimentos infantiles (potitos) categorizadas con el atributo de ecológicos. Analizar las contribuciones a las Ingestas Dietéticas de Referencia (IDR) de estos micronutrientes a partir del consumo de estos alimentos infantiles (Capítulos 3 y 4).

Objetivo 4: Determinar la bioaccesibilidad de elementos inorgánicos (Ca, Mg, Fe, Zn, Cu, Mn, Se y Cd) presentes en las formulaciones de potitos ecológicos anteriores, su contenido en otros macronutrientes (proteínas, grasa y fibra) y la influencia de éstos últimos en los valores de bioaccesibilidad de elementos inorgánicos obtenidos. Valoración nutricional y toxicológica de los potitos ecológicos frente a los convencionales (Capítulos 3 y 4).

Capítulos

3. **CAPÍTULOS**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AP

3.1. CAPÍTULO 1: Peas and lentils

AM Ramírez Ojeda, Universidad de Córdoba, Córdoba, Spain

Introduction

Legumes are a food group consisting of nearly 19000 species consumed by both humans and animals due to their low cost, high protein contents, complex polysaccharides, and minerals. However, legumes also contain other substances that may lower its nutritional value, such as enzyme inhibitors, oxalates, phytates, and polyphenols. Mediterranean countries in particular are noted for their high consumption of legumes in the form of beans, chickpeas, peas, and lentils, usually available in two forms – dry and overcooked. Furthermore, lentils in particular are highly valued in the countries of Southern Europe. All varieties that are grown and produced in these countries are of the species Lens esculenta.

About a third of the lentils produced worldwide is in India and consumed in the Indian domestic market. The biggest consumers are in Asia, North Africa, Western Europe, and parts of Latin America. Canada is the largest exporter of lentils in the Americas, with the main production region being Saskatchewan. The Palouse region of Eastern Washington and the Idaho Panhandle region, with their collective commercial center in Pullman (WA), constitute the largest lentil-producing region of the United States. Algeria and Egypt are the largest importers of lentils, followed by Bangladesh, Sri Lanka, India, and Pakistan. In Europe, the largest importers are Spain, France, Italy, and Germany. Finally, in Latin America, the countries of the Andean Community and Brazil all imported about 140000 tonnes of lentils.

The FAO reported the world production of lentils in 2008 at 3.874 million tonnes, with the largest producer being India (with 29.0% of global production), followed by Canada (24.5%), Turkey (14.5%), Australia (5.7%), the United States (4%), and China (3.9%), together accounting for 81% of the global production of lentils. The National Agricultural Statistics Service (NASS) reported that in 2007, US production was 154500 tonnes in North Dakota, Montana, Washington, and Idaho combined. Canada estimated its 2009–10 production to be a record 1.5 million tonnes.

In the case of peas, they are produced in almost every country in the world. Canada is the largest producer (about 25% of total world production) and the main exporter (40% of total world exports) of dry peas in the world. More than half of all Canadian pea production is exported as seed without subject to any processing. About 10% of the peas produced are used as seed for crops. Whole and cracked seeds are also used for stews and soups and as canned food. The cover is added to breads rich in fiber; however, most peas become animal feed. The largest use of dried peas in Europe and North America is in the food industry, where whole seeds are ground and mixed with cereal flours to produce food products. The main use of peas in Asia and South America is for human consumption, where the whole or cracked seeds are first boiled and then eaten.

Carbohydrates

The concentrations of total carbohydrates found in lentil flour and pea flour are between 625 and 657 mg g^{-1} dry matter with a higher concentration in peas than in lentils. Among these carbohydrates, starch stands out as the major component, with percentages of between 59.1% and 53.6%, respectively, of the total carbohydrate content. However, the digestibility of this starch is affected by many factors and is generally low. Among these factors, we can include firstly granule size, which is mainly defined by the relationship between the surface area and volume. As the grain size increases, the contact between the substrate and amylases decreases and thereby the rate of enzymatic digestibility ($r=0.791$, $p<0.05$). This result is consistent with the fact that peas, with an average granule size of about 20 mm, present a lower digestibility than lentils with starch granule diameters of about 17.5 µm.

A further factor that also influences the digestibility of starch is the degree of crystallization of the granule. Starch consists of amylose chains, a linear polymer made of glucose units linked through α-1,4, and amylopectin chains and a branched polymer consisting of glucose units linked by 1,4 bonds α-bonded to the branching main chain by α-1,6 glycosidic bonds. These chains may be in the amorphous phase or the crystalline phase. The higher the content of amylopectin in the structure of the starch granule, the higher the crystallinity ($r=0.914$, $p<0.01$). Similarly, the presence of amylose reduces this crystallization degree ($r=0.775$, $p<0.05$). It is expected therefore that the existence of a higher crystallinity in starch granules will decrease the rate of enzymatic digestion by hindering access to glycolytic enzyme substrate. Accordingly, several studies have shown an increased presence of amylose in peas and lentils, which justifies its rapid hydrolysis.

This starch digestibility can be improved by cooking treatment. Thus, it has been observed that the digestibility can alternate between 37% and 42% in the case of raw legumes, 70–77% for the standard cooked variation (100 ºC for 90 min), and even 87–91% with autoclaving at 121 ºC for 10 min. However, it is not apparent that increasing the cooking time leads to enhanced starch digestibility. This improvement in the digestibility of starch could be attributed to hydrolysis as a result of heat treatment and the destruction and removal of antinutritional substances, such as phytic acid and tannins, thus creating more space in the matrix, increasing the susceptibility to enzymatic attack. Heat treatment can also decrease the percentage of resistant starch from 2.4% to 1.9% for peas and from 3.3% to 2.5% for lentils.

The fraction of starch that is not hydrolyzed by amylases after approximately 2 h resists enzymatic digestion and becomes part of the dietary fiber. Germination treatment can also decrease the resistant starch content by reducing the concentration of α-amylase inhibitors.

Fiber

Dietary fiber is a concept widely used to refer to a complex mixture of indigestible polysaccharides including cellulose, hemicelluloses, oligosaccharides, pectins, gums, waxes, and lignin commonly found in the cell walls of plant cells. The properties of dietary fiber in relation to human nutrition are associated with distinct physiological responses including viscosity, water-holding capacity, fermentability, bulk, and the ability to bind bile acids.

Dietary fiber can be classified into two groups based on solubility in a pH-controlled enzyme solution, each one with distinct physiological properties and nutritional benefits. Insoluble fiber is mainly related to intestinal transit, thereby improving laxation. In addition to this, insoluble fiber is fermented in the large intestine by promoting the growth of flora in the intestine (prebiotic effect). The formation of short-chain fatty acids originating from the fermentation process can also prevent the development of colon tumor cells. Moreover, the soluble dietary fiber can help to lower blood cholesterol (hypocholesterolemic effect) and regulate blood glucose levels. Several human studies have shown that the inclusion of pea fibers can decrease blood glucose peak. Pea fibers have been also used in the treatment of hypercholesterolemic patients.

The total dietary fiber content ranges between 180 and 350 g kg^{-1} for peas and 90 and 300 g kg^{-1} for lentils. Within this content, the highest percentage is represented by the insoluble fraction (about 70% of total dietary fiber) comprising cellulosic glucose, poor lignin, and xyloglucans (predominant hemicelluloses in dicotyledonous plants). Arabinoses (also known as rich pectins) are abundant in the soluble group of dietary fibers.

Another important group of compounds of dietary fiber also found in significant concentrations in lentils, peas, and other pulses is α-galactooligosaccharides, also known as the raffinose family oligosaccharides.

Finally, with regard to simple carbohydrates, the following sugars have been identified in the flour of lentils and peas: monosaccharides (0.085–0.124 fructose, 0.042 glucose, and mainly 0.722 galactose and 0.305–0.521 ribose g/100 g) and disaccharides (0.647–0.697 sucrose, 0.039–0.191 maltose and 0.159 melibiose g/100 g). Saccharose concentrations were also analyzed with results between 20.8 and 31.9 mg g^{-1} in dry matter. Also found in oligosaccharides is alpha-galactoside and within this, the family of rafinosas. But in this last group, the links between monosaccharides are produced by links α-1,2, which cannot be digested by humans, becoming part of dietary fiber as discussed in the succeeding text.

These carbohydrates are characterized by intestinal gas production in humans, that is, cause flatulence consumption, because the gastrointestinal tract does not synthesize α-galactosidase, the enzyme acting on these oligosaccharides. High concentrations of raffinose, stachyose, and verbascose were found in raw lentils (RL).

Processed lentils and peas can reduce the concentration of these oligosaccharides responsible for flatulence. Soaking them for 3–12 h can show reductions of up to 75% of content. Thus, it appears as if soaking can reduce the raffinose family oligosaccharides and ciceritol by 75% in cooked lentils and peas soaked for 3–12 h. Furthermore, cooking also reduces levels of saccharose, raffinose, and stachyose. Germination can also cause a decrease in these substances that results in an increase in the activity of alpha-galactosidase.

It has been shown that an addition of commercial α-galactosidase enzyme or endogenous action to pea flour and lentil flour reduces the same raffinose, stachyose, and verbascose substantially (from 41% to 100%), as well as ciceritol in lentils.

It has also been shown that both culture and the environment have an effect on the sugar content of lentil seeds. Thus, different concentrations of raffinose family oligosaccharides between cultivars may be due to differences between biosynthetic enzyme and raffinose family oligosaccharides. However, there is so far no comparative study. It has been observed that the total concentration of lentil-cultured raffinose family oligosaccharides may also vary according to different environmental conditions. According to the results shown, it can be ascertained that lentils, which receive less precipitation, show higher content of raffinose family oligosaccharides compared with those who received more precipitation in their environment. However, no conclusive correlation between the surface area in which they were grown and the concentration of raffinose family oligosaccharides was obtained.

Proteins

Legumes have a high protein content, with lentils recording between 22% and 31% and peas between 26% and 32% (expressed as dry matter). Yet, despite this high protein content, its quality is not as high as proteins of animal origin. It should be noted that there are factors that influence the lower digestibility of these proteins, such as the presence of protease inhibitors and very compact quaternary structures, which make difficult the action of enzymes. Similarly, some authors observe the existence of noncovalent interactions or disulfide bonds between the proteins as being associated with the fraction of insoluble dietary fiber.

Another factor that affects the quality of protein in peas and lentils is their deficiency in many essential amino acids. With regard to the content of amino acids, it has been found that RL and peas are rich in aspartic acid, glutamic acid, asparagine, and arginine. However, as with other legumes, they are deficient in sulfuric amino acids, such as methionine, cysteine, and tryptophan. Therefore, the combination of vegetables (which contain a high proportion of lysine compared with cereals) and foods rich in sulfuric amino acid, such as cereals, is a common practice that provides all essential amino acids. This combination increases the nutritional quality of protein available so that it is similar to the values found in animal proteins.

As is the case for starch, the nutritional value of these proteins can also be increased during culinary preparation, because protease inhibitors are inactivated by heat and other structural changes occurring that facilitate access to the enzymes, thus promoting digestive absorption of proteolysis and nitrogen compounds.

Germination treatment may also increase the protein content of free amino acids depending on the circumstances in which it is conducted. With respect to lentils, it has been observed that in the presence of light during germination, higher amounts of free amino acids and protein–protein amino acids may occur (with some exceptions), which would not be found if germinated in darkness. However, in peas, the opposite effect has been observed. It has also been found that germination time affects the content of free amino acid proteins in legumes differently. Thus, a study shows that in lentils, germination time primarily increases the content of serine, asparagine, glycine, threonine, proline, and valine. In peas, the amino acid contents that are increased over time are proline, histidine, and tyrosine. In both peas and legumes, the content of other amino acids, such as lysine, over time decreases during germination, in both the presence and the absence of light. These changes in the amino acid content during germination could be related to the hydrolysis of proteins, synthesis, and reorganization.

Germination involves the mobilization of reserves of protein in the cotyledons, along with the synthesis of new proteins for shoot growth. The new proteins can be composed of various amino acids stored in protein, altering the amino acid content pattern with respect to the raw legume. Furthermore, amino acids produced by the hydrolysis of protein reserves are not only used exclusively for the synthesis of new components but also used as an energy source, especially in the early stages of germination.

Furthermore, the results indicate that bleaching, sterilization, and storage of peas show no effects on the protein content, compared with that of fresh peas. Nor are losses observed in the total protein content in bottled or canned peas stored under different conditions (10 ºC and room temperature) for a period of 6 months. However, a slight variation is produced in the total amino acid in legumes. The results suggest that Maillard reactions or other reactions occurred, including hydroperoxides formed from occurring unsaturated fats.

Lipids

As with many plant foods, lentils and peas have a low fat content ranging from 0.7% to 6.1%. When it comes to the lipid profile of RL, mono- and polyunsaturated fatty acids –unsaturated oleic, linoleic, and linolenic – stand out at a rate of about 20%, 38%, and 7%, respectively, compared with the total of fatty acids. The contents of saturated fatty acids, more atherogenic potential, are about 0.5%, 18%, and 2% for myristic, palmitic, and stearic fatty acids, respectively. Similarly, a fatty acid profile present in raw peas has a content of 13% oleic acid, 35% linoleic acid, and 5.5% linolenic acid. The percentage of saturated fatty acids is approximately 22%, highlighting palmitic acid (14%). In the case of canned peas, with a heat treatment applied prior to being cooked, linoleic acid remains the principal fatty acid.

In some varieties of peas, it has been observed that this fatty acid profile may vary depending on the size of the seed. In the early stages of growth (peas with a size of between 4.7–7.5 mm and 7.6–8.2 mm), the ratio of unsaturated to saturated fatty acids increases slightly due to a series of changes in enzyme systems involved in the biosynthesis of fatty acids. Afterward (pea size of 8.3–8.8 mm), this ratio stabilizes and finishes by declining sharply in the final stage (pea size of 8.9–10.2 mm). Accordingly, some industries order these peas for the manufacture of flours, as this latter size would be the required size in order to decrease the susceptibility to autoxidative deterioration.

The content of fatty acids increases during storage because of the lipolysis of triglycerides, polar lipids, and sterol esters, although a higher temperature of storage favors all these processes. It has been observed in red lentils that the percentage of free fatty acids can reach up to six times its original levels within 16 weeks at a storage temperature of 40 ºC, while storage temperatures at 10 or 20 ºC show no increase in fatty acids. Meanwhile, the moisture content of lentils also influences this lipolysis of fat as a decrease of moisture content from 17.5% to 12.5% increases the content of free fatty acids by a factor of 3, rather than 6 at 40 ºC. Furthermore, the material in which they are packaged also changes the conditions of all these aforementioned processes. When packaged in glass jars, the concentration of free fatty acids is significantly higher than in a container of lacquered tinplate. While this influence of packing material has not been sufficiently studied, it is possible that the transparent nature of the glass containers favors the action of light on lipolysis from fat.

Trace Elements

Many legumes, such as lentils and peas, have relatively high levels of phosphorus, magnesium, iron, zinc, and copper. However, the mineral bioavailability of pulses is generally poor, as a result of its low digestibility, high content in dietary fiber, or antinutritional components, such as phytic acid, oxalates, and polyphenols, all of which can interfere with mineral absorption. Thus, an *in vitro* study to determine mineral bioaccessibility, using a simulated gastrointestinal tract, found iron dialyzability percentages of 2.6% for chickpeas and lentils. In the case of zinc, zinc dialyzability percentages were better, being 32.2% and 48.5% for chickpeas and lentils, respectively.

Phytate is the major storage form of phosphorus in legumes. The inhibitory effect of phytate on the absorption of minerals in the small intestine is due to the formation of insoluble mineral–phytate complexes. It has been found that phytate concentrations ranged between 0.06 and 0.33 g per 100 g for peas and between 0.08 and 0.30 g per 100 g for lentils, which causes high content but is still however lower than those found in beans (between 0.34 and 0.58 g per 100 g). However, the negative effect of phytic acid on mineral bioavailability depends on the amount of phytic acid present in the legume as a molar ratio of phytate/mineral. Phytate/zinc molar ratios below 5 are designated as high zinc availability, ratios within the range of 5–15 as moderate availability, and ratios above 15 as low availability. In the case of iron, phytate/ iron molar ratios are recommended as being lower than 1 for good bioavailability. A phytate/mineral molar ratio for zinc and iron of around 18 and 2.1 in lentils and 22 and 6.5 in peas, respectively, has been reported, which is in accordance with the low rates of mineral bioavailability found in these legumes.

Oxalate salts are very insoluble at intestinal pH, and it has been widely documented that oxalic acid decreases the absorption of minerals in monogastric animals. The oxalate content of cooked peas ranged between 6 and 4 g per 100 g and in lentils, around 8 g per 100 g. Although the concentration of antinutritional substances is again lower than those determined in other legumes such as beans (85–26 g per 100 g), also, it is expected that this oxalate content affects the decrease in its bioaccessibility in the intestinal lumen.

Peas and lentils are also rich sources of polyphenols – natural antioxidants that can replace synthetic antioxidants used in food. It has been observed that the seed coat of lentils has a higher content of flavonoid compounds than cotyledon that is primarily rich in nonflavonoid compounds. The ability of the polyphenols to decrease mineral bioavailability has been studied mainly with regard to iron and calcium. It has been observed, however, that not all polyphenols have the same capacity to reduce mineral absorption. Thus, several studies suggest that flavonoids and their polymers do not interfere with iron absorption in humans, whereas polyphenols with a high galloyl ester content exhibit a potent inhibitory effect. In this sense, it can be seen that these areas of study need to be further researched.

Different studies have evaluated the effect of a series of treatments such as soaking, cooking, extrusion, and germination to reduce the inhibitory capacity of phytic acid, oxalate, and polyphenols on mineral bioavailability. These treatments can promote or inhibit the development of phytases, significantly affecting the hydrolysis of phytic acid.

One study shows that during the soaking, all phenolic components identified in peas and lentils decrease drastically. This decrease was due to leaching of water-soluble phenols into the soaking water. Likewise, traditional cooking of lentils (cooking by boiling (the ordinary method)) can reduce the concentration of tannins and phytic acid by up to 25%. Cooking lentils in the autoclave results in a reduction of 33–46% for tannins and 28–52% for phytic acids, with losses increasing in line with increased cooking time (10–90 min) and increases in temperature (121–128 ºC). Meanwhile, the germination of lentils and peas increases the activity of endogenous enzymes (such as hydrolases, polyphenol oxidases, and phytases) resulting in a reduction of phenolic compounds and degradation of phytic acid forms with a lower content of phosphate groups, as inositol tri- and tetraphosphates with less chelating ability. Various *in vitro* and *in vivo* studies show that these treatments improve the mineral bioavailability of these legumes.

Phytic acid can also be reduced by endogenous phytase activity, which works by breaking inositol hexaphosphate into different forms that possess less mineral chelating ability, thus leading to an increase in iron dialyzability resulting from this enzymatic dephytinization in lentils. Similar results were found for pea flour, with enhanced iron absorption after treatment with exogenous microbial phytase, leading to inositol hexaphosphate levels below those reported by other authors.

A study using an exogenous microbial phytase in optimum conditions (pH 5.5 and 37 ºC) shows dephytinization and subsequent removal in the soaking solution after transformation and the effect on iron bioavailability meal in pea (*Pisum sativum* L.). This experiment was conducted on rats by examining the chemical composition of pea flour and the digestive and metabolic utilization of the mineral mentioned.

The rats were fed with three types of diets: raw pea meal, raw pea meal that underwent soaking treatment without phytase, and raw pea meal that underwent soaking treatment with phytase.

Soaking pea flour leads to a considerable reduction in the iron content (33%), while a smaller reduction in the iron content (7%) is observed, associated with a higher total phosphorus concentration that is obtained with an additional phytase treatment. The digestive utilization by growing rats of iron from raw pea meal and soaked peas was negligible but increased significantly as a result of treatment with phytase. The low absorption of iron obtained by the first two dietary treatments during an experimental period of 10 days was not reflected in any hematologic indexes (red cell count, hemoglobin, and hematocrit) or tissues (the femur, heart, and kidney) studied, with the exception being the sternum.

Finally, an *in vivo* study using growing rats observed a significant increase in digestive calcium utilization as a result of lentils being germinated for 6 days.

A total of 40 rats were divided into four groups of ten, and each group was fed with one of the four types of diets: control, diet containing casein (20%) + methionine (0.5%), the tested diet with raw lentils (RL), and diet with dry-heated lentils (HL) and germinated lentils (GL).

It has been reported that diets containing lentils provide a lower quality of the protein casein, due to lentils being deficient in sulfuric amino acids. The macropeptides that may be formed during consumption reduce calcium absorption. Therefore, a low absorption of calcium in sprouted lentils was expected, because the protein quality is lower when compared with RL or HL. However, this increased absorption can be explained due to the possible effects of germination on other factors such as fiber, phytic acid, and oxalic acid.

The results show a drastic drop in hemicellulose. This indicates that it is possible to achieve maximum absorption of calcium when lower hemicellulose content in the diet was found. Also, the content of inositol hexaphosphoric acid or phytic acid (IHP) was decreased compared with HL. It is possible that this decrease plays an important role, since the absorption of calcium in sprouted lentils was 20% higher than in animals fed with RL. Furthermore, rats fed with GL also showed a major transformation of phytic acid. It has been shown that calcium bioavailability is related to the levels of phytic acid. In GL, the transformation of phytic acid increased significantly, which can be translated into a greater total phosphorus content, expressed as a percentage of total phosphorus present as IHP (% IHP-P/total P), following the reduction of 26% of IHP per g of dry matter. Thus, the results show increased calcium absorption in sprouted lentils (value of 82) that had a lower content of IHP, compared with diets of HL.

It has been shown that the Ca/P ratio affects the absorption of Ca. A low Ca/P ratio observed in RL and HL compared with lentils from the control diet can be a cause of the low absorption of calcium (see Table 1).

Table 1 Mineral contents (mg/100 g)

	Lentils				*Peas*			
Minerals	*Raw*	*Soaked*	*Cooked*	*Germinated*	*Raw*	*Soaked*	*Cooked*	*Germinated*
Ca	42–97.3	19–69	15.4–50.21	86–94	55–91	85.9	14	NE
P	380–541	203–418	180–462	283–318	295–335	317–365	99	NE
Mg	129	45–115	36–118	NE	115–132	117–129	109–120.5	NE
Zn	3.6–5.11	NE	1.27–3.13	4.77–5.49	2.67–3.68	2.37–3.16	2.71–3.7	NE
Cu	0.852–1	NE	0.25–0.94	NE	0.87–6.33	0.94–5.87	0.181–0.78	NE
K	753–905	295–752	422–520	NE	981	861–909.5	488–599	NE
Fe	7.5–9.02	NE	1.29–2.4	9.03–11	7.36	3.56–4.96	1.29–4.8	NE
			3.3		1.5–5.2			

NE: Not examined.

Vitamins

Different literature sources show lentils and peas as good sources of soluble B vitamins, especially niacin and pantothenic acid. However, they are deficient in vitamin C, carotene, and retinol. With respect to folic acid, although readily occurring in natural sources, the folic acid is not actually available due to binding with other biomolecules to the form complexes.

It has been shown that concentrations of thiamine, riboflavin, and niacin can be more changed during processing than before consumption. Study shows a percentage reduction in the content of thiamine (54–62%) and niacin (25–61%) during soaking and cooking of lentils and peas, with the percentage of losses being greater the higher the rate of hydration.

However, one study shows that germination produces no significant change in the content of thiamine in lentils and peas, while an increase in the content of riboflavin of between 20% and 135% for lentils and from 60% to 166% in peas is observed – possibly due to an increase in microbial activity.

These changes in the vitamin content of these legumes can be explained by several factors. In relation to losses during soaking, these are easily accountable, based on their solubility in water soaking, with the loss being greater the weaker the strength of its link to cell structures. Accordingly, thiamine (consisting of a pyrimidine ring and a thiazole ring interconnected by a methylene bridge) and niacin (in the form of nicotinic acid and/or nicotinamide) are simpler chemical molecules with a higher water solubility than riboflavin – this is associated with complex molecular structures such as flavin mononucleotide and flavin adenine dinucleotide, which account for the lower solubility. Cooking also increases the loss of these water-soluble vitamins as a result of high rates of leaching due to the high cooking temperatures achieved and the increase in levels of sodium ions (see Table 2).

Table 2 Vitamin contents (mg/100 g)

	Lentils				Peas			
Vitamins	*Raw*	*Soaked*	*Cooked*	*Germinated*	*Raw*	*Soaked*	*Cooked*	*Germinated*
Thiamine	0.427–0.439	0.38–0.412	0.162–0.212	0.48–0.53	0.70–0.78	NE	0.19	0.68–0.78
Riboflavin	0.03–0.41	0.102–0.121	0.056–0.072	0.24–0.49	0.15–0.22	NE	0.056	0.23–0.40
Niacin	0.88–2.06	0.427–0.924	0.405–0.929	NE	2.89–3.15	NE	0.89	NE
Folic acid	0.03–1.5 433	NE	181	NE	101.5	21	24.5–25.1	NE
Vitamin A	17–112 39 IU	NE	8 IU	NE	149 IU	NE	7 IU	NE
Vitamin E	0.33–0.62	NE	0.11	NE	0.3–1.02	NE	0.39	NE
Vitamin C	ND 7	NE	1.5	NE	1.8–40	NE	0.4	NE

Antinutritional Substances

Despite the potential nutritional value of pulses as a relatively inexpensive source of significant amounts of protein, carbohydrates, vitamins, and minerals, the use of these as a food source has been limited due to the presence of certain antinutritional factors. These include the presence of phytic acid, condensed tannins, and polyphenols, as discussed in the preceding text, plus protease inhibitors (trypsin and chymotrypsin) and α-amylase inhibitors, which reduce the digestibility of proteins and carbohydrates.

Protease Inhibitors

Protease inhibitors have been extensively investigated in legumes, as they may have an important impact on the nutritional value of these pulses. Protease inhibitors may inhibit the function of digestive enzymes, mainly acting on trypsin and chymotrypsin (it may affect other enzymes although to a lesser extent). This results in affecting the digestive utilization of the protein, causing pancreatic hypertrophy, and instigating weight loss and nitrogen removal in feces.

Although the values of trypsin inhibitor activity were observed in both peas and lentils, these are lower than those in other legumes. Thanks to their character, these thermolabile protease inhibitors may be reduced by standard cooking procedures of seeds, such as soaking, or conventional low-pressure cooking. One study shows how the roasting and boiling of flour, lentils, and peas affect the activity of trypsin inhibitor. The roasting and boiling processes reduced the activity of trypsin inhibitor; a maximum of 95.6% reduction was found in roasted shelled green lentils, while the lowest (37.8%) was found in the flour of boiled shelled yellow peas. The effects of roasting do not significantly differ from those of boiling, except for red lentils with husks, which had the lowest values of trypsin inhibitor activity after roasting compared with those after boiling.

Furthermore, it has been shown that during the soaking process, certain losses are produced in the trypsin inhibitor activity as a result of the leaching process. In soaked peas and lentils, this treatment can produce a reduction of trypsin inhibitor by up to 15.4%. Variations in the percentage of losses of the inhibitor can be attributed to changes in the permeability of the seed shell that allows antinutritional solubilization of this substance in the soaking liquid. Germination for 72 h can also potentially reduce the occurrence of trypsin inhibitor by between 58% and 76%. However, treatment by extrusion processing produces a greater reduction (about 95%).

Inhibitors (α-Amylase)

α-Amylase plays an important role in the metabolism of carbohydrates, by hydrolyzing the type of linkages between monosaccharides and providing glucose as an energy source for humans and animals. It has been shown that the inhibitor reduces α-amylase digestion of starches, thus reducing postprandial glucose, and increases the circulation of insulin. This can be beneficial in treatments for obesity or diabetes mellitus; however, in other cases, it decreases the nutritional and energy values of these foods.

Soaking was observed to have reduced the activity of alphaamylase inhibitor. This may be due to leaching in the steepwater. Similarly, germination for 72 h was also found to have reduced the activity of α-amylase inhibitor by up to 48%; this reduction may become a complete extrusion treatment.

See also: Phytic Acid: Properties, Uses, and Determination; Pulsed Electric Fields.

Further Reading

Campos-Vega, R., Loarca-Pina, G. and Oomah, B. D. (2010). Minor components of pulses and their potential impact on human health. Food Research International 43: 461–482.

De, J., Berrios, J., Morales, P., Cámara, M. and Sánchez-Mata, M. C. (2010). Carbohydrate composition of raw and extruded pulse flours. Food Research International 43: 531–536.

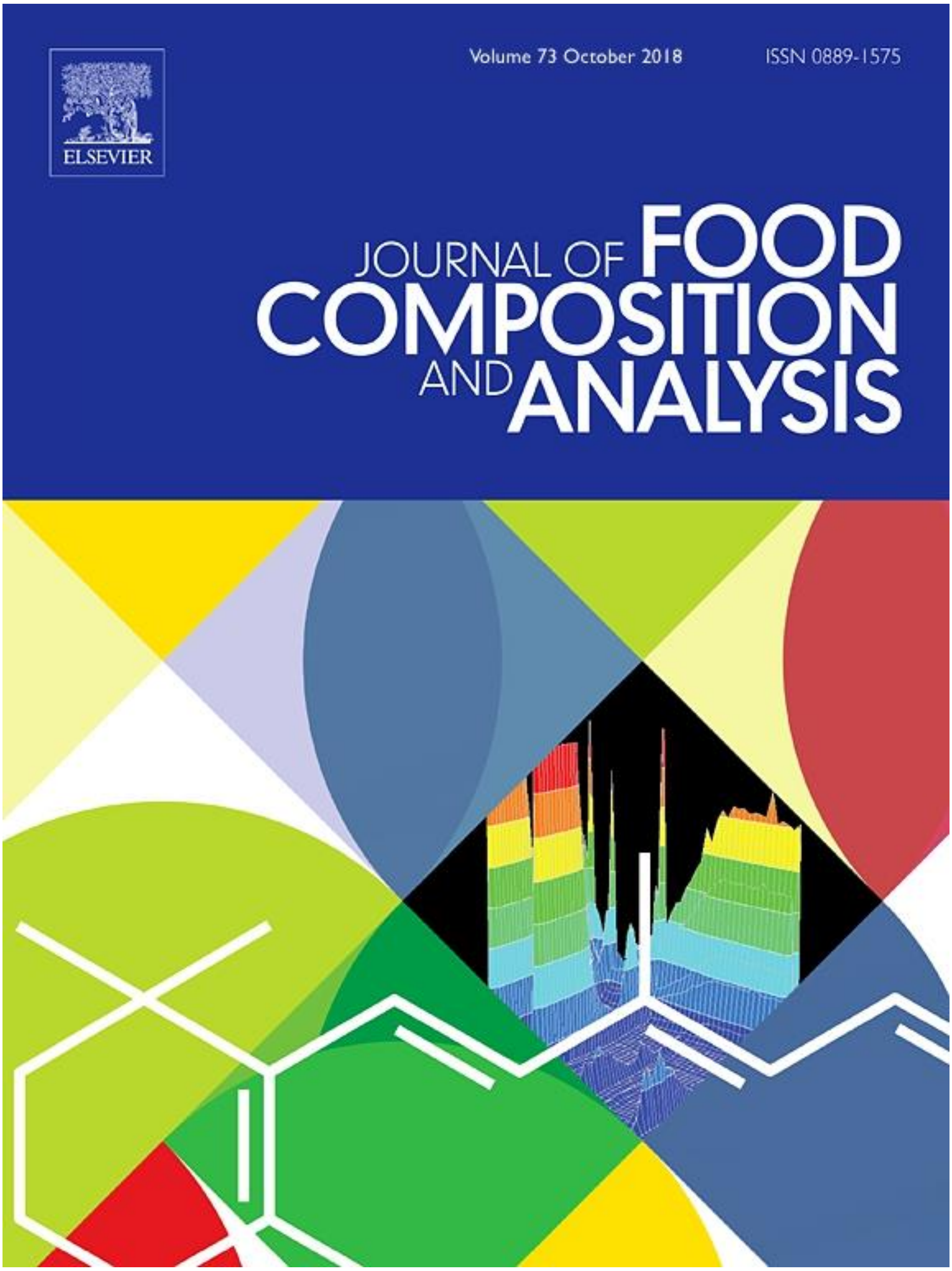
Kuo Y-H, Rozan P, Lambein F, Frias J, and Vidal-Valverde C (2004) Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes. Food Chemistry 86: 537–545.

Sandberg A-S (2002) Bioavailability of minerals in legumes. British Journal of Nutrition 88(Suppl. 3): S281–S285.

Relevant Websites

http://en.wikipedia.org/wiki/Legume – Legumes.

http://www.mercasa.es/ – Mercasa.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3.2. CAPÍTULO 2: Mineral and trace element content in legumes (lentils, chickpeas and beans): bioaccesibility and probabilistic assessment of the dietary intake

AM. Ramírez-Ojeda, R. Moreno-Rojas, and F. Cámara-Martos*

Departamento de Bromatología y Tecnología de los Alimentos, Campus Universitario de Rabanales, Edificio C-1, 14014, Córdoba, Spain

***Author for correspondence:** Prof. Fernando Cámara-Martos, PhD. Departamento de Bromatología y Tecnología de los Alimentos, Universidad de Córdoba. Campus Universitario de Rabanales, Edificio Charles Darwin C-1, 14014, Córdoba, Spain.

Tel.: + 34 957 212 026

Fax: + 34 957 212 000

e-mail: bt2camaf@uco.es

Abstract

Three samples of legumes widely consumed in Spain (lentils, beans and chickpeas) were selected in order to assess the total and bioaccessible content of trace elements. The influence of dietary components and the effect of processing on elements bioaccessibility were also evaluated. Raw lentils presented medium contents of 84, 34, 13, 9.1, 450 and 922 µg g^{-1} for Fe, Zn, Mn, Cu, Ca and Mg respectively. Raw chickpeas and beans presented the following contents, 45, 37, 26, 8.3, 828, 1176 µg g^{-1} and 45, 24, 12, 6.9, 977, 1166 µg g^{-1}. Results showed that element total content decreased in cooked samples with respect to soaked ones, due to a solubilization of the inorganic elements into the water during cooking treatments at different rates. Thus, trace element concentrations differed, resulting in the following: lentils (10, 5.2, 1.8, 1.8, 219, 155 µg g^{-1}); chickpeas (9.1, 7.2, 4.3, 2.9, 297, 273 µg g^{-1}) and beans (11, 5, 2.1, 1.8, 312, 268 µg g^{-1}). However, elements initially present are considerably more bioaccessible probably due to a destruction of some antinutritional components as a consequence of processing. Finally, according to a probabilistic assessment used to determine the contributions to Dietary References Intakes (DRI), legumes were proper sources of Fe, Cu, Mn and Zn.

Keywords: Legumes, Lentils, Chickpeas, Beans, Trace element, Mineral, Bioaccessibility, DRI, Food analysis, Food composition.

1. Introduction

Legumes have been, and in some areas still are, one of man's basic foodstuffs. These basic crops have been an integral part of the human diet for millennia, but today legumes are not experiencing anywhere near the same increase in production as corn, wheat, rice and soybeans, and its consumption has undergone a slow, but constant, decline in both developed and developing countries (FAO, 2016). From a nutritional point of view, they are a good source of vegetable proteins and amino acids, fiber, and at the same time, are low in fats (Torija and Díez, 1999; Olmedilla et al., 2010; Iqbal et al., 2006; Khattab and Arntfield, 2009; Tharanathan and Mahadevamma, 2003). Moreover, legumes may also constitute an appropriate source of proteins for cattle (Jezierny et al., 2010) and similarly, a good source of minerals and trace elements such as Fe and Zn (Jodral-Segado and Navarro-Alarcón, 2003; Campos-Vega et al., 2010). As a result of the above reasons, the General Assembly of the United Nations, at its 68th session, proclaimed 2016 as the International Year of Pulses (A / RES / 68/231). This year it was proposed to raise the public's awareness about the nutritional benefits of legumes as part of sustainable food production with the aim of achieving food security, combating malnutrition, reducing poverty, improving human health and increasing agricultural sustainability (FAO, 2016).

In Spain, three species of legumes stand out for their high consumption rates: beans (*Phaseolus vulgaris* L.), chickpeas (*Cicer arietinum* L.) and lentils (*Lens culinaris* L.). The most notable consumption is associated with chickpeas (1.3 kg per person per year), while beans and lentils reach 0.9 kg per person in each case (Mercasa, 2015). Most legumes of dietary significance (such as beans, chickpeas and lentils) are widely marketed in dried and/or processed forms (ready-to-eat). In the case of the dried form, the traditional way of legume preparation includes soaking them in water followed by cooking, a treatment expected to alter their macro- and micronutrient composition. They are usually consumed boiled as part of a stew. In relation to processed forms - ready-to eat products - they are previously cooked in industrial machinery and packed in glass jars. Subsequently, they can be consumed as part of a salad, side dish or stew.

From a nutritional point of view, it would be very useful to know the concentrations of several micronutrients, such as mineral or trace elements, present in this food group. In this regard, previous studies have already been developed with different types of legumes (Akinyele and Shokunbi, 2015; Campos-Vega et al., 2010; Iqbal et al., 2006; Cabrera et al., 2003). However, only determining the content of minerals or trace elements present in a food may not be sufficient to evaluate its nutritional quality. It is much more appropriate to assess the portion of this element, initially present in the food, which is solubilized and absorbed in the intestinal lumen. Later, this fraction will be used by the body for the physiological function for which it is intended, thus resulting in the concept of bioaccessibility (Cámara-Martos et al., 2015).

In this sense, studies to determine bioaccessibility of inorganic elements in legumes mainly focus on Fe, Zn and Ca (Singh et al., 2016; Ramírez-Cárdenas et al., 2010; Viadel et al., 2006a, b; Lombardi-Boccia et al., 2003). However, to our knowledge, no study has been conducted to analyze the bioaccessibility of other elements such as Mn, Cu and Mg, which are in abundance in pulses. These micronutrients are also needed for proper development of an organism and their deficiency leads to a number of pathologies (Avila et al., 2016; Bertinato and Finglas, 2016; Mazur and Maier, 2016). Furthermore, the bioaccessibility of these elements may be altered by several dietary components and the effect of processing (Ramírez-Ojeda et al., 2016; Cámara et al., 2007; Viadel et al., 2006a, b; Cámara et al., 2005; Sebastiá et al., 2001).

Given the above, the objectives of this article were i) to determine the bioaccessibility and the total content of Fe, Zn, Mn, Cu, Ca and Mg present in three varieties of legumes (lentils, beans and chickpeas) widely consumed by the Spanish population, sold in two formats (soaked raw and ready-to-eat); ii) to study the influence of processing and other food components such as protein and fat in the bioaccessibility of these elements; iii) to assess the contributions to the Dietary Reference Intakes (DRIs) of these micronutrients, from data obtained by consumption of these legumes, through a probabilistic approach.

2. Materials and methods

2.1 Materials and reagents

Deionized water obtained with a Milli-Q system (Millipore, Madrid, Spain) was used exclusively. To eliminate the risk of contamination, all polyethylene material and glassware, after each use, were washed with tap water, soaked in a 20% HNO_3 solution (at least overnight), and rinsed with deionized water three times.

All reagents were of analytical-reagent grade. To obtain working standards, standard solutions of Cu, Zn, Mn, Fe, Ca and Mg (1000 mg/L) (Scharlau Chemie, Barcelona, Spain) were used and diluted as necessary. High quality concentrated nitric acid (HNO_3) 65%, and hydrochloric acid (HCl) 35% (Panreac, Barcelona, Spain) were used for sample mineralization. Sodium bicarbonate ($NaHCO_3$) 97% was supplied by Scharlau (Barcelona, Spain). Lanthanum chloride ($LaCl_3$) was obtained from Perkin Elmer (Madrid, Spain).

Sigma-Aldrich Co. (St. Louis, MO) provided the digestive enzymes (pepsin; pancreatin) and bile salts. 3.2 g of pepsin (P-7000 from porcine gastric mucosa) were dissolved in 20 mL of HCl (0.1 M) to prepare the pepsin solution. The solution of pancreatin and bile salts was obtained by dissolving in 150 mL of 0.1 M $NaHCO_3$, 0.6 g of pancreatin (P-3292 from porcine pancreas) and 3.9 g of bile salts (B-8756 of porcine origin). The working solutions were prepared immediately before use. The dialysis membranes, with a pore size (MWCO) of 12–14,000 Å (Size 6 Inf Dia 27/32"–21.5 mm, 30 m, Bestlno. 1063F09, Medicell Int. LTD, London, UK), were rinsed several times with distilled deionized water before use.

2.2 Samples

Lentils, chickpeas and beans of three different brands, widely sold in Spain, were chosen. Each different brand was selected in both a raw as well as ready-to-eat format. In the latter case, legumes were directly analyzed (previous preparation of sample), while in the case of the raw samples, they were soaked in deionized water, at room temperature overnight, before use in the study. Three different packets from different supermarkets, representing both raw and ready-to-eat formats of pulse, were used in several periods of this study (September 2015 to March 2016). In total, the number of legumes used in the laboratory was 54 (3 legumes × 3 brands × 3 batches × 2 formats available for purchase). All samples were poured onto Petri dishes, freeze-dried and packed in polypropylene vacuum bags, until required for analyses.

2.3. Total mineral content

1 g of a ground and lyophilized sample was ashed for 15 h in a muffle furnace at 460 °C, to determine the total content of mineral (Moreno-Rojas et al., 1994). Once the ash was cooled, this was bleached with 2.5 mL of HNO_3 2 N, dried on thermostatic hotplates, until complete mineralization, for 1 h in a muffle furnace at 460 °C. Afterwards, the ash was dissolved in a 1 mL solution of HCl 20% (v/v) and made up to a known volume (10 mL) with deionized water. Each sample was analyzed in quintuplicate. Therefore, the number of samples analyzed was 270 (54 legumes · 5).

Atomic absorption spectrometry (FAAS) with a Varian SpectraAA – 50B model (Palo Alto, California, USA), equipped with standard air-acetylene flame and single element hollow cathode lamps, was used to determine Zn, Fe, Ca and Mg content. In the case of Ca and Mg, $LaCl_3$ was added to the mineral solution at a final concentration of 2%, to avoid interference by phosphate. Electrothermal atomic absorption spectrometry (ETAAS) was used for the determination of Cu and Mn in a soluble and dialyzable fraction by a Perkin-Elmer model Analyst 600 (Waltham, Massachusetts, USA) with graphite furnace and an autosampler. The instrumental conditions for the determination are shown in Tables 1 and 2. The detection limit (LOD) was calculated as the mean value of 30 measurements of the blank plus three times their standard deviation. Regarding the quantification limit (LOQ), it was calculated as the mean value of 30 measurements of the blanks plus 10 times their standard deviation. These parameters are registered in Table 1. Furthermore, CRM (NIST, Gaithersburg, Maryland, USA) were also analyzed (under the same conditions as samples) to evaluate the accuracy of the proposed method. The selected materials were SRM 1568 (rice flour) (NIST, Gaithersburg, Maryland, USA) and BCR 185R (bovine liver) (EVISA, Germany). Values obtained are in good agreement with the certified values (Table 1).

Table 1
Instrumental conditions, limit of detection, limit of quantification and analysis of certified references materials.

Element	Wavelength (nm)	Slit Width (nm)	LOD ($mg\ L^{-1}$)	LOQ ($mg\ L^{-1}$)	Certified references material ($mg\ kg^{-1}$)					
					Rice flour NIST – 1568a			Bovine liver BCR – 185R		
					Certified	Found	Recovery (%)	Certified	Found	Recovery (%)
Fe	248.3	0.2	0.084	0.28	7.42 ± 0.44	7.58 ± 0.52	102	-	-	
Zn	213.9	0.7	0.168	0.56	19.42 ± 0.26	20.38 ± 0.24	105	138.6 ± 2.1	130.4 ± 17.1	94
Mn	279.5	0.2	0.013	0.043	19.20 ± 1.80	18.48 ± 4.20	96	11.07 ± 0.29	11.32 ± 2.87	102
Cu	324.8	0.7	0.014	0.05	2.35 ± 0.16	2.26 ± 0.34	96	277 ± 5	264 ± 38	95
Ca	422.7	0.7	0.315	1.05	118.4 ± 3.1	116.7 ± 2.1	98	-	-	
Mg	285.2	0.7	0.011	0.036	559 ± 10	556 ± 18	99	-	-	

LOD limit of detection, *LOQ* limit of quantification

Table 2.
Instrumental operating parameters for Cu and Mn ET – AAS determination

Step	T (ºC)	Ramp Time (s)	Hold Time (s)	Internal Flow (mL/min)
Drying	110	15	20	250
Pyrolysis	900	10	20	250
Atomization	2000	0	4	0
Cleaning	2600	1	3	250

2.4. Procedure for in vitro gastrointestinal digestion

2.4.1.Solubility assay:

To determine trace element or mineral solubility, the procedure used was that described by Cámara et al. (2005). Therefore, we proceed as follows: the quintuplicate samples of 3 g powdered of soaked raw or ready-to-eat were homogenized with 20 mL of HCl 0.1 N. The content was adjusted to pH 2 using HCl 6 N. Then, a pepsin–HCl acid solution (0.125 g of porcine pepsin by 3 g of lyophilized sample) was added to each mixture and incubated at 37 °C in a shaker water bath for 120 min (HSB-2000 Shaking Bath; E-Chrom Tech CO. LTD, Taipei, Taiwan). After the incubation, the pH of the samples was adjusted to 5 using $NaHCO_3$ 1 M. This was followed by addition of a mixture of pancreatin and bile salts (0.025 g of pancreatin and 0.160 g of bile salts by 3 g of lyophilized sample) to the flask's content and incubated for a further 120 min under the same conditions. Following this, the pH was adjusted to 7.2 with NaOH 0.5 M. The contents were centrifuged at 4000 rpm for 60 min (Eppendorf Centrifuge 5810 R). The supernatants were collected, its organic matter was destroyed in a muffle furnace at 460ºC, and the mineral content was calculated by atomic absorption spectrometry.

2.4.2.Dialyzability assay:

The ground samples (5g) were weighed in flasks, resuspended with 20mL HCl 0.1N and subjected to simulated gastric digestion by incubation with pepsin. This step was similar to that in the above assay (the assays were developed by quintuplicate for each sample). After the gastric digestion step, segments of dialysis tubing (molecular mass cut-off 12-14000 Å) containing a solution of sodium bicarbonate in concentrations equimolar to sodium hydroxide, as determined by titratable acidity, were inserted into the gastric digest (Cámara – Martos et al. 2005). After 1h, digested samples were treated with $NaHCO_3$ to bring pH to 5, then, the pancreatic-bile salt mixture was added and incubation was continued in a thermostatic shaker

(37ºC) for 2h more to simulate intestinal digestion. Afterward, digested samples were quantitatively transferred to quartz crucibles, and heated until complete mineralization. The mineral and trace element content present in dialyzable fraction was quantified by atomic absorption spectrophotometry.

2.5. Protein, fat content determinations

AOAC methods were used to determine the proximate composition: defatting in Soxhlet apparatus with petroleum ether for crude fat and micro-Kjeldahl for protein. Briefly, to describe Kjeldahl method, 0.5 g of lyophilized sample with a catalyst pellet were placed in a digestion flask and 20 mL of concentrated sulfuric acid was added. The mixture was then heated in a digester until the solution cleared. After cooling, 70 mL of deionized water were added, and a small quantity of NaOH, which converted the ammonium salt to ammonia. The ammonia gas was led into a trapping solution of HCl 0.1N and determined by back titration with a NaOH solution 0.1N. The protein concentration was calculated from the nitrogen values using a conversion factor of 6.25. For the determination of fat content, a 3–5 g of lyophilized sample was weighed and wrapped in a paper filter and subsequently transferred into a Soxhlet liquid/solid extractor with petroleum ether for 1 h. After fat extraction, samples were dried in desiccators and weighed.

2.6 Statistics and probabilistic assessment

The data were analyzed using SPSS 15.0 (IBM, Armonk, NY). In order to validate the normality of the data obtained, the Shapiro-Wilks test was used. Later, Pearson's correlation (parametric conditions) and Spearman's correlation (non-parametric conditions) were used for determining the dependence between variables. Significant differences were considered when $p<0.05$.

A probabilistic model was developed to estimate the intake level for Fe, Zn, Mn, Cu, Ca and Mg derived from consumption of legumes (lentils, chickpeas and beans interchangeably). The model here developed followed a probabilistic approach in which variables were described by probability distributions. They were fitted to concentration data obtained in our study for each element (total element concentration and bioaccessible element concentration). Furthermore, in order to estimate the intake level, serving size was considered. It was assumed a serving size ranging from 150 to 200 g for adult population, which was defined by a uniform distribution in the probabilistic model; meaning that all values in that range had the same probability to occur.

The probability distributions describing the Fe, Zn, Mn, Cu, Ca and Mg concentration data were fitted by using a commercial software, @ Risk v7.5 (Palisade, Newfield, NY, USA). The simulation was run using 100,000 interations for each element. The goodness of fit to data was assessed by using different statistical tests which corresponded to Akaike Information Criterion (AIC) test and Chi-square test. These statistical tests allow researchers to give a guess of how well the fitted distribution described the observed data. In addition, the visual analysis was equally considered to assess the fit of the probability distributions to intake data.

3. Results and discussion

Table 3
Total and bioaccesible iron (soluble and dialyzed) analyzed in legumes (µg · g^{-1} fresh matter; mean ± standar desviation)

			Soaked			Cooked		
LENTIL		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	88 ± 5	38 ± 2	6.4 ± 0.9	4.1 ± 2.0	7.1 ± 0.3	5.1 ± 0.0	4.1 ± 2.6
	Batch 2	84 ± 6	36 ± 2	6.5 ± 1.2	2.7 ± 0.9	12 ± 1	5.5 ± 0.2	3.2 ± 0.7
	Batch 3	91 ± 12	43 ± 6	6.1 ± 1.4	3.6 ± 0.8	7.1 ± 0.6	3.7 ± 0.5	3.1 ± 0.9
Brand 2	Batch 1	108 ± 7	51 ± 3	8.1 ± 0.4	3.8 ± 1.6	7.8 ± 0.3	8.2 ± 3.2	2.2 ± 1.2
	Batch 2	91 ± 4	47 ± 2	8.0 ± 2.3	1.8 ± 0.9	7.7 ± 0.3	6.6 ± 0.5	2.8 ± 1.0
	Batch 3	107 ± 34	45 ± 14	5.0 ± 2.2	1.7 ± 0.4	13 ± 1	6.4 ± 0.8	2.4 ± 0.7
Brand 3	Batch 1	58 ± 6	28 ± 3	5.0 ± 2.6	3.0 ± 1.9	14 ± 1	9.4 ± 0.4	2.5 ± 0.2
	Batch 2	59 ± 4	28 ± 2	4.2 ± 0.1	2.2 ± 1.0	13 ± 1	6.3 ± 1.9	3.3 ± 0.5
	Batch 3	69 ± 4	29 ± 2	4.6 ± 0.9	1.8 ±1.1	11 ± 0	5.5 ± 1.1	2.3 ± 0.3
Average value		84 ± 19	38 ± 8.8	6.0 ± 1.4	2.7 ± 0.9	10 ± 3	6.3 ± 1.7	2.9 ± 0.6
CHICKPEA		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	49 ± 2	22 ± 1	12 ± 3	3.3 ± 1.3	7.2 ± 1.6	10 ± 2	3.6 ± 1.0
	Batch 2	44 ± 2	18 ± 1	6.8 ± 0.0	3.0 ± 0.9	8.3 ± 0.8	7.0 ± 0.5	4.8 ± 1.0
	Batch 3	41 ± 3	19 ± 1	7.4 ± 0.9	2.5 ± 0.6	4.4 ± 1.4	7.2 ± 0.6	4.0 ± 0.6
Brand 2	Batch 1	34 ± 1	18 ± 1	7.9 ± 0.5	2.3 ± 0.9	8.2 ± 0.6	8.3 ± 0.7	4.1 ± 1.6
	Batch 2	50 ± 3	25 ± 2	7.9 ± 3.6	2.4 ± 1.1	12 ± 0	9.7 ± 0.2	4.6 ± 2.1
	Batch 3	50 ± 3	20 ± 1	5.0 ± 1.8	2.4 ± 1.0	9.6 ± 0.5	8.9 ± 0.9	3.7 ± 0.5
Brand 3	Batch 1	46 ± 3	22 ± 1	6.6 ± 1.1	3.0 ± 1.6	10 ± 1	14 ± 1	3.7 ± 1.1
	Batch 2	40 ± 2	19 ± 1	6.3 ± 1.1	2.8 ± 2.1	12 ± 0	6.6 ± 0.1	5.1 ± 1.8
	Batch 3	50 ± 8	21 ± 3	6.7 ± 1.8	3.5 ± 2.0	10 ± 1	8.7 ± 0.3	3.4 ± 0.9
Average value		45 ± 6	20 ± 2	7.4 ± 2.0	2.8 ± 0.4	9.1 ± 2.4	8.9 ± 2.2	4.1 ± 0.6
BEAN		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	48 ± 1	21 ± 1	10 ± 1	3.5 ± 1.2	13 ± 1	8.1 ± 1.0	3.4 ± 0.6
	Batch 2	44 ± 5	20 ± 2	11 ± 2	4.6 ± 1.2	7.6 ± 0.3	5.5 ± 0.4	4.4 ± 1.1
	Batch 3	46 ± 3	22 ± 2	8.3 ± 1.8	5.1 ± 0.8	3.9 ± 1.0	6.0 ± 0.1	2.5 ± 0.7
Brand 2	Batch 1	47 ± 3	22 ± 2	8.6 ± 0.6	3.9 ± 0.9	13 ± 1	7.5 ± 0.2	4.1 ± 0.9
	Batch 2	43 ± 3	20 ± 1	9.9 ± 0.4	2.8 ± 1.3	11 ± 1	9.9 ± 0.3	3.8 ± 0.3
	Batch 3	42 ± 3	22 ± 2	10 ± 2	4.5 ± 1.5	12 ± 1	15 ± 0	5.7 ± 0.8
Brand 3	Batch 1	49 ± 3	24 ± 2	11 ± 0	2.5 ± 1.0	16 ± 1	18 ± 3	4.6 ± 0.3
	Batch 2	42 ± 3	19 ± 1	8.4 ± 0.5	2.8 ± 1.1	12 ± 1	6.5 ± 0.9	3.7 ± 1.1
	Batch 3	42 ± 4	20 ± 2	9.2 ± 1.9	3.0 ± 1.5	9.2 ± 0.1	7.7 ± 0.2	2.9 ± 0.6
Average value		45 ± 3	21 ± 2	9.7 ± 1.2	3.6 ± 0.9	11 ± 4	9.4 ± 4.4	3.9 ± 0.9

Table 4
Total and bioaccesible zinc (soluble and dialyzed) analyzed in legumes (µg · g-1 fresh matter; mean ± standar desviation

			Soaked			Cooked		
LENTIL		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	41 ± 4	18 ± 2	9.6 ± 0.7	2.7 ± 0.3	3.1 ± 0.2	3.5 ± 0.1	1.3 ± 0.2
	Batch 2	44 ± 4	19 ± 2	11 ± 1	2.1 ± 0.4	7.4 ± 0.2	3.7 ± 0.4	1.8 ± 0.3
	Batch 3	41 ± 2	17 ± 1	11 ± 0	2.7 ± 0.5	3.8 ± 0.1	2.9 ± 0.6	1.5 ± 0.1
Brand 2	Batch 1	47 ± 3	19 ± 1	11 ± 0	3.0 ± 0.2	3.8 ± 0.3	4.0 ± 0.4	1.0 ± 0.1
	Batch 2	29 ± 2	15 ± 1	9.6 ± 1.9	3.7 ± 0.3	4.2 ± 0.1	3.6 ± 0.6	0.93 ± 0.17
	Batch 3	15 ± 14	15 ± 6	7.0 ± 1.3	2.9 ± 0.2	5.1 ± 0.1	4.0 ± 0.8	1.4 ± 0.4
Brand 3	Batch 1	26 ± 3	12 ± 1	6.9 ± 0.7	1.9 ± 0.6	8.1 ± 0.8	5.0 ± 1.	2.0 ± 0.3
	Batch 2	25 ± 3	12 ± 1	7.2 ± 0.9	3.0 ± 0.5	5.9 ± 0.3	3.3 ± 0.5	1.7 ± 0.1
	Batch 3	36 ± 1	15 ± 0	7.0 ± 0.2	2.3 ± 0.3	5.2 ± 0.1	4.0 ± 0.7	1.8 ± 0.1
Average value		34 ± 11	16 ± 3	9.0 ± 2.0	2.7 ± 0.5	5.2 ± 1.7	3.8 ± 0.6	1.5 ± 0.4
CHICKPEA		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	49 ± 7	22 ± 3	8.0 ± 0.9	2.3 ± 0.5	7.0 ± 0.2	6.5 ± 0.8	2.0 ± 0.1
	Batch 2	45 ± 6	19 ± 3	8.7 ± 0.3	1.8 ± 0.1	8.4 ± 0.4	5.9 ± 0.1	2.0 ± 0.4
	Batch 3	76 ± 18	32 ± 8	9.1 ± 0.1	2.4 ± 0.2	3.2 ± 0.3	5.5 ± 0.5	2.0 ± 0.6
Brand 2	Batch 1	26 ± 2	11 ± 1	5.8 ± 0.1	1.7 ± 0.2	7.8 ± 0.1	5.3 ± 0.8	1.6 ± 0.7
	Batch 2	26 ± 3	13 ± 1	5.9 ± 1.1	2.6 ± 0.2	6.2 ± 0.2	5.0 ± 0.2	1.3 ± 0.5
	Batch 3	28 ± 1	11 ± 1	5.6 ± 0.8	1.8 ± 0.1	6.2 ± 0.2	4.7 ± 0.2	1.7 ± 0.1
Brand 3	Batch 1	34 ± 3	16 ± 1	6.6 ± 0.1	2.4 ± 0.2	9.6 ± 1.9	5.8 ± 0.4	2.0 ± 0.4
	Batch 2	26 ± 1	12 ± 0	6.1 ± 1.1	2.3 ± 0.4	9.5 ± 0.2	5.3 ± 0.4	1.7 ± 0.2
	Batch 3	28 ± 2	11 ± 1	6.8 ± 0.8	2.2 ± 0.3	6.9 ± 0.5	4.7 ± 0.3	1.6 ± 0.5
Average value		37 ± 17	16 ± 7	7.0 ± 1.3	2.2 ± 0.3	7.2 ± 2.0	5.4 ± 0.6	1.8 ± 0.2
BEAN		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	37 ± 9	16 ± 4	9.5 ± 0.3	1.3 ± 0.1	5.3 ± 0.1	5.0 ± 0.1	1.7 ± 0.2
	Batch 2	21 ± 3	9.7 ± 1.0	8.3 ± 0.7	1.8 ± 0.5	4.2 ± 0.2	3.4 ± 0.2	2.0 ± 0.3
	Batch 3	26 ± 2	13 ± 1	6.4 ± 0.0	1.9 ± 0.3	3.6 ± 0.1	2.7 ± 0.6	1.2 ± 0.1
Brand 2	Batch 1	23 ± 2	10 ± 1	6.3 ± 0.7	1.8 ± 0.4	5.2 ± 0.2	3.6 ± 0.5	1.5 ± 0.3
	Batch 2	23 ± 2	12 ± 1	5.0 ± 0.6	2.3 ± 0.5	4.5 ± 0.1	16 ± 3	1.5 ± 0.2
	Batch 3	24 ± 2	11 ± 1	5.9 ± 0.4	2.4 ± 0.5	4.5 ± 0.4	5.2 ± 0.0	2.0 ± 0.4
Brand 3	Batch 1	23 ± 1	11 ± 1	4.8 ± 0.5	2.6 ± 0.1	6.6 ± 0.5	3.8 ± 0.5	1.6 ± 0.2
	Batch 2	22 ± 2	9.8 ± 1.1	4.2 ± 0.7	2.5 ± 0.4	5.3 ± 0.2	3.8 ± 0.1	1.5 ± 0.1
	Batch 3	21 ± 2	10 ± 1	4.8 ± 0.5	2.4 ± 0.5	4.6 ± 0.0	3.1 ± 0.2	1.4 ± 0.2
Average value		24 ± 5	11 ± 2	6.2 ± 1.8	2.1 ± 0.4	5.0 ± 0.9	5.2 ± 4.1	1.6 ± 0.3

3.1. Lentils

Raw lentils provided medium amounts of Fe and Zn (84 and 34 µg/g, respectively) in fresh matter, (Tables 3 - 4) which is in agreement with amounts found in previous studies (Cabrera et al., 2003; Viadel et al., 2006a, b; Wang and Daun, 2006; Campos-Vega et al., 2010). The effect of soaking meant that the values of these trace elements fell to 38 and 16 µg/g of fresh matter for Fe and Zn, respectively, due to water uptake during overnight soaking. Nevertheless, when mineral and trace element concentrations are expressed in dry matter, analyzed concentrations for all elements studied varied scarcely between raw and soaked samples, suggesting that there is no migration of elements to water soaked (data not shown). According to the results, it can be pointed out that lentils are an important source of Fe and Zn, especially if we compare these values with some kind of meat, such as cured pork loin or raw partridge (37 and 40 µg/g of Fe respectively) (Ortega et al., 2004) and raw pork sirloin or raw sirloin beef (16 and 18 µg/g of Zn respectively) (Chan et al., 1995). In relation to the rest of the elements studied, concentrations in raw lentils ranged between 6 – 11, 9 – 17, 387 – 490, 808 – 1092 µg/g, for Cu, Mn, Ca and Mg respectively (Tables 5 – 8). These values were similar in the case of Cu (8 – 11 µg/g), Mn (10 – 15 µg/g) and Mg (993 – 1090 µg/g), but were lower for Ca (592 – 762 µg/g), than those reported by Wang et al. (2009).

With respect to studied elements' bioaccessibility (solubility assay) in soaked lentils, Zn and Ca, showed the highest percentages, with 26.9% and 20.0% respectively. On the other hand, Mn and Fe were the elements with the lowest solubility percentage (13.1% and 7.1%, respectively). This tendency is repeated in the dialyzability assays with Ca and Zn, showing the highest percentages (9.5% and 8.0%, respectively), while Fe and Mn had the lowest (3.3% and 3.0%, respectively) (see Tables 3 – 8). Another study has also reported high solubility percentages for Zn and Ca (53.6% and 28.8%) and Fe the lowest (11.5%) in lentils (Sahuquillo et al., 2003). A positive correlation was also observed between total Cu and Fe soluble ($r = 0.825$; $p < 0.01$) and between Fe soluble and Cu dialyzed ($r = 0.667$; $p < 0.05$) for soaked lentils, demonstrating a certain positive interaction between Cu and Fe in relation to its bioaccessibility. This synergistic effect between both elements has been also reported in other *in vitro* studies with a food matrix such as weaning foods (Ramírez – Ojeda et al., 2016) and school menus (Cámara et al., 2007). The mechanism for this positive interaction remains unclear, however this strong correlation between both elements has just been reported for *in vivo* models as due to the influence of Cu on Fe metabolism and hemoglobin biosynthesis (Harris, 2001; Collins et al., 2010; Gulec and Collins, 2014; Ha et al., 2016). On the other hand, a positive interaction was also observed between soluble Fe and total Zn ($r = 0.668$; $p < 0.05$).

This synergistic effect between Fe and Zn has also been documented in previous studies (Yamaji et al., 2001; Iyengar et al., 2012; Ramírez – Ojeda et al., 2016). However, the contrary effect has already been reported by other authors (O´Brien et al., 2000; Chung et al., 2002; Troost et al., 2003; Cámara et al., 2007; Hemalatha et al., 2009) which seems to indicate that this effect may depend on the concentration to which these elements are initially found. Nevertheless, the high content of Fe present in soaked lentils showed a negative correlation upon the dialyzed Ca concentration ($r = -0.884$; $p < 0.01$) and the dialyzed Mg concentration ($r = -0.758$; $p < 0.05$).

Furthermore, comparing mineral concentrations in dry matter between soaked and industrial cooked lentils for all branches and batches studied, it was observed that the total mineral content decreased in cooked samples with respect to soaked ones, due to minerals and trace elements being leached from the lentil seeds into the water during cooking treatments, at different rates (see Tables 3 – 8). The more significant reduction was observed in the case of Fe, with a medium decrease of 55%, followed by Mn (52%), Zn (48%), Mg (39%) and Cu (29%). Previous studies have also observed similar reductions for the elements studied when a traditional or an industrial cooking method is applied (Viadel et al., 2006a; Hefnawy, 2011). Contrary to this, it should also be noted that Ca content increased during the industrial cooking treatment (78.2%). Viadel et al (2006a) has also pointed to a similar increase of this mineral in lentils when an industrial cooking method is used. This observed effect is difficult to account for and several studies should be undertaken to determine the correct cause of this phenomenon.

With respect to the bioaccessibility of cooked lentils (solubility and dialyzability assays), it was observed that cooking had a positive effect on most elements studied. Although the elements' concentration present in cooked lentils is lower than in soaked ones, the inorganic elements initially present are considerably more bioaccessible (see Tables 3 – 8). It has been widely documented that certain antinutritional components, such as phytic acid, oxalates, antitrypsin inhibitors, and polyphenols, can interfere with mineral absorption (Mohan et al., 2016; Johnson et al., 2013; Campos-Vega et al., 2010; Thavarajah et al., 2010). Thus, phytic acid with its phosphate groups, can chelate divalent ions (such as Ca, Mg and particularly Fe and Zn) due its strong affinity towards these elements (Vashishth et al., 2017). On the other hand, oxalates bind to Ca by creating complexes that are poorly soluble in the intestinal lumen (Mohan et al., 2016). However, the cooking treatment has been proven to be an effective method to reduce or remove some of these dietary components from the pulses (Shi et al., 2018; Máñez et al., 2002; Wang et al., 2009; Hefnawy, 2011). As a consequence, Fe and Zn were the elements which showed the highest solubility and dialyzability percentages (73.1% and 29.1%; 60.5% and 27.7%, respectively). Similarly, high bioaccessibility percentages for Zn (69.9 and 25.3 for solubility and dialyzability) have also been found in stewed lentils (Cámara et al., 2005). Similarly to what occurred with raw lentils, a statistically significant correlation between total Fe and total Zn (r = 0.833; $p < 0.01$), and between soluble Fe and soluble Zn (r = 0.854; $p < 0.01$) in cooked lentils was observed, which reinforces the positive interaction between both elements in relation to their bioaccessibility. On the other hand, the lowest mineral bioaccessibility percentages for solubility and dialyzability, respectively, were found in the case of Cu (43.9% and 27.3%) and Ca (31.4% and 10.9%). Similar values for Cu and Ca dialyzability (25.0 and 10.3) have been reported by Cámara et al (2005). On the other hand, a positive correlation between soluble Ca concentration and soluble Mg concentration (r = 0.800; $p < 0.01$) must be highlighted, which reveals a synergistic interaction between both elements.

Also, cooked lentils stood out for their high protein values, which ranged from 21 to 29 g/100 g and low lipids content, which ranged from 0.77 to 2.64 g/100 g (Table 9). This data is in line with that reported by other authors (de Almeida Costa et al., 2006; Iqbal et al., 2006; Wang and Daun, 2006; Wang et al., 2009). For this reason, it can be highlighted that lentils could be considered an important vegetable protein source, (Genovese & Lajolo, 2001), and thus suitable for vegetarian diets or diet regimens.

Table 5
Total and bioaccesible copper (soluble and dialyzed) analyzed in legumes (mg · Kg^{-1} fresh matter; mean ± standar desviation).

			Soaked			Cooked		
LENTIL		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	11 ± 1	4.5 ± 0.5	1.5 ± 0.1	0.40 ± 0.18	1.5 ± 0.3	0.72 ± 0.43	0.59 ± 0.24
	Batch 2	9.5 ± 0.6	4.0 ± 0.2	1.6 ± 0.1	0.26 ± 0.05	1.7 ± 0.2	0.67 ± 0.02	0.55 ± 0.09
	Batch 3	10 ± 2	4.7 ± 0.8	1.3 ± 0.0	0.34 ± 0.10	0.98 ± 0.18	0.79 ± 0.08	0.50 ± 0.04
Brand 2	Batch 1	11 ± 2	5.1 ± 0.7	1.5 ± 0.1	0.55 ± 0.06	1.5 ± 0.1	1.1 ± 0.1	0.38 ± 0.05
	Batch 2	9.1 ± 0.5	4.7 ± 0.3	1.9 ± 0.5	0.55 ± 0.07	1.7 ± 0.1	0.70 ± 0.08	0.41 ± 0.12
	Batch 3	10 ± 1	4.3 ± 0.6	1.6 ± 0.1	0.47 ± 0.07	2.5 ± 0.1	1.1 ± 0.2	0.51 ± 0.08
Brand 3	Batch 1	6.3 ± 0.1	3.0 ± 0.1	1.7 ± 0.2	0.38 ± 0.38	2.3 ± 0.3	0.75 ± 0.07	0.47 ± 0.06
	Batch 2	5.8 ± 0.6	2.7 ± 0.3	1.3 ± 0.1	0.16 ± 0.15	2.1 ± 0.2	0.37 ± 0.20	0.51 ± 0.06
	Batch 3	8.6 ± 1.0	3.6 ± 0.4	1.1 ± 0.0	0.36± 0.05	2.0 ± 0.1	0.94 ± 0.32	0.51 ± 0.07
Average value		9.1 ± 1.9	4.1 ± 0.8	1.5 ± 0.3	0.39 ± 0.13	1.8 ± 0.4	0.79 ± 0.23	0.49 ± 0.06
CHICKPEA		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	10 ± 1	4.5 ± 0.3	2.3 ± 0.0	0.22 ± 0.07	3.4 ± 0.3	0.58 ± 0.43	0.47 ± 0.09
	Batch 2	9.6 ± 0.9	4.0 ± 0.4	1.9 ± 0.0	0.27 ± 0.11	2.8 ± 0.4	0.61 ± 0.25	0.45 ± 0.08
	Batch 3	9.6 ± 0.4	4.1 ± 0.2	1.4 ± 0.2	0.27 ± 0.05	2.4 ± 0.1	0.61 ± 0.10	0.37 ± 0.08
Brand 2	Batch 1	8.8 ± 1.1	4.3 ± 0.5	1.6 ± 0.1	0.25 ± 0.05	2.9 ± 0.2	0.39 ± 0.05	0.43 ± 0.09
	Batch 2	7.6 ± 0.6	3.8 ± 0.3	1.7 ± 0.2	0.40 ± 0.07	2.8 ± 0.5	0.68 ± 0.09	0.33 ± 0.02
	Batch 3	8.9 ± 0.5	3.5 ± 0.2	1.7 ± 0.0	0.29 ± 0.06	2.1 ± 0.3	0.68 ± 0.06	0.33 ± 0.02
Brand 3	Batch 1	7.7 ± 0.9	3.6 ± 0.4	1.9 ± 0.2	0.54 ± 0.15	3.7 ± 0.2	0.41 ± 0.08	0.31 ± 0.06
	Batch 2	6.2 ± 0.4	2.4 ± 1.1	1.9 ± 0.4	0.29 ± 0.10	3.4 ± 0.1	0.36 ± 0.10	0.27 ± 0.04
	Batch 3	5.9 ± 1.2	2.5 ± 0.5	2.0 ± 0.1	0.23 ± 0.05	2.6 ± 0.3	1.0 ± 0.1	0.28 ± 0.14
Average value		8.3 ± 1.5	3.6 ± 0.7	1.8 ± 0.3	0.31 ± 0.10	2.9 ± 0.5	0.59 ± 0.20	0.36 ± 0.07
BEAN		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	8.1 ± 1.2	3.6 ± 0.5	1.8 ± 0.1	0.35 ± 0.13	2.0 ± 0.4	0.74 ± 0.23	0.38 ± 0.09
	Batch 2	6.3 ± 0.9	2.9 ± 0.4	1.1 ± 0.2	0.49 ± 0.12	1.4 ± 0.2	0.39 ± 0.21	0.52 ± 0.17
	Batch 3	9.9 ± 0.5	4.8 ± 0.3	2.5 ± 0.3	0.44 ± 0.16	0.57 ± 0.08	0.47 ± 0.02	0.33 ± 0.07
Brand 2	Batch 1	5.7 ± 1.0	2.8 ± 0.4	1.7 ± 0.3	0.31 ± 0.04	2.3 ± 0.1	0.48 ± 0.30	0.53 ± 0.07
	Batch 2	6.6 ± 0.5	3.5 ± 0.3	1.7 ± 0.2	0.47 ± 0.10	1.7 ± 0.1	0.61 ± 0.16	0.37 ± 0.08
	Batch 3	6.9 ± 0.5	3.2 ± 0.2	1.7 ± 0.1	0.39 ± 0.03	1.7 ± 0.3	1.0 ± 0.0	0.74 ± 0.08
Brand 3	Batch 1	6.6 ± 0.6	3.3 ± 0.3	1.6 ± 0.2	0.08 ± 0.02	2.6 ± 0.2	0.87 ± 0.08	0.51 ± 0.10
	Batch 2	5.5 ± 0.4	2.5 ± 0.2	1.7 ± 0.1	0.10 ± 0.02	1.9 ± 0.3	0.97 ± 0.67	0.37 ± 0.06
	Batch 3	6.8 ± 0.3	3.3 ± 0.2	1.9 ± 0.3	0.28 ± 0.13	1.9 ± 0.2	0.70 ± 0.25	0.33 ± 0.03
Average value		6.9 ± 1.3	3.3 ± 0.7	1.8 ± 0.4	0.32 ± 0.15	1.8 ± 0.6	0.69 ± 0.22	0.45 ± 0.14

Table 6
Total and bioaccesible manganese (soluble and dialyzed) analyzed in legumes (µg · g-1 fresh matter; mean ± standar desviation).

				Soaked			Cooked	
LENTIL		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	16 ± 2	6.7 ± 0.7	2.0 ± 0.1	0.45 ± 0.04	1.6 ± 0.2	0.77 ± 0.05	0.36 ± 0.15
	Batch 2	14 ± 1	5.9 ± 0.3	3.8 ± 0.3	0.48 ± 0.02	3.2 ± 0.2	1.5 ± 0.2	0.54 ± 0.06
	Batch 3	17 ± 1	7.9 ± 0.5	2.1 ± 0.4	0.65 ± 0.05	0.65 ± 0.15	1.4 ± 0.1	0.49 ± 0.03
Brand 2	Batch 1	15 ± 1	6.6 ± 0.4	1.7 ± 0.8	0.18 ± 0.04	1.8 ± 0.2	1.2 ± 0.1	0.33 ± 0.02
	Batch 2	8.7 ± 0.4	4.5 ± 0.2	0.92 ± 0.25	0.20 ± 0.05	1.7 ± 0.1	0.46 ± 0.07	0.35 ± 0.07
	Batch 3	12 ± 3	4.9 ± 1.2	1.5 ± 0.2	0.10 ± 0.02	2.0 ± 0.1	0.72 ± 0.05	0.43 ± 0.09
Brand 3	Batch 1	14 ± 1	6.5 ± 0.3	1.6 ± 0.1	0.53 ± 0.05	1.5 ± 0.2	1.2 ± 0.0	0.29 ± 0.02
	Batch 2	12 ± 2	5.7 ± 0.8	1.0 ± 0.2	0.63 ± 0.05	1.9 ± 0.1	0.66 ± 0.46	0.33 ± 0.03
	Batch 3	13 ± 0	5.3 ± 0.2	1.2 ± 0.3	0.42 ± 0.02	1.6 ± 0.1	0.71 ± 0.04	0.28 ± 0.01
Average value		13 ± 2	6.0 ± 1.0	1.8 ± 0.9	0.40 ± 0.20	1.8 ± 0.6	0.96 ± 0.37	0.38 ± 0.09
CHICKPEA		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	28 ± 2	12 ± 1	6.7 ± 0.3	0.76 ± 0.04	4.8 ± 0.9	1.9 ± 0.0	0.68 ± 0.03
	Batch 2	47 ± 2	20 ± 1	5.0 ± 0.9	0.81 ± 0.06	5.1 ± 0.7	1.4 ± 0.1	0.72 ± 0.08
	Batch 3	11 ± 5	15 ± 2	3.0 ± 0.9	0.88 ± 0.06	1.0 ± 0.1	2.8 ± 0.2	0.84 ± 0.27
Brand 2	Batch 1	31 ± 1	15 ± 1	2.7 ± 0.2	0.41 ± 0.06	4.4 ± 0.1	2.2 ± 0.3	0.74 ± 0.24
	Batch 2	27 ± 2	12 ± 1	1.9 ± 0.3	0.50 ± 0.09	5.0 ± 0.6	1.1 ± 0.1	0.75 ± 0.43
	Batch 3	19 ± 2	7.2 ± 1	1.7 ± 0.1	0.30 ± 0.09	3.1 ± 0.4	1.0 ± 0.1	0.79 ± 0.04
Brand 3	Batch 1	22 ± 3	10 ± 1	2.1 ± 0.4	0.48 ± 0.17	5.7 ± 0.8	3.3 ± 0.2	0.81 ± 0.13
	Batch 2	26 ± 3	12 ± 1	1.3 ± 0.8	0.51 ± 0.05	5.5 ± 0.3	1.7 ± 0.1	0.71 ± 0.07
	Batch 3	25 ± 2	9.5 ± 1	0.83 ± 0.1	0.42 ± 0.12	4.5 ± 0.6	1.5 ± 0.1	0.54 ± 0.11
Average value		26 ± 10	13 ± 4	2.8 ± 1.9	0.56 ± 0.20	4.3 ± 1.5	1.9 ± 0.7	0.73 ± 0.09
BEAN		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	15 ± 1	6.8 ± 0.4	2.5 ± 0.2	0.64 ± 0.04	3.2 ± 0.6	1.6 ± 0.0	0.59 ± 0.04
	Batch 2	12 ± 1	5.6 ± 0.4	1.7 ± 0.5	0.72 ± 0.02	1.8 ± 0.0	0.70 ± 0.11	0.68 ± 0.09
	Batch 3	12 ± 1	5.6 ± 0.5	0.75 ± 0.10	0.44 ± 0.00	0.35 ± 0.11	1.0 ± 0.1	0.45 ± 0.03
Brand 2	Batch 1	12 ± 1	5.3 ± 0.4	0.93 ± 0.16	0.49 ± 0.04	2.4 ± 0.1	1.1 ± 0.3	0.70 ± 0.05
	Batch 2	11 ± 1	5.0 ± 0.4	1.4 ± 0.2	0.53 ± 0.02	2.5 ± 0.0	0.89 ± 0.04	0.93 ± 0.08
	Batch 3	12 ± 1	5.8 ± 0.6	1.4 ± 0.3	0.66 ± 0.01	1.9 ± 0.3	0.91 ± 0.30	1.0 ± 0.1
Brand 3	Batch 1	12 ± 1	5.9 ± 0.4	0.95 ± 0.24	0.60 ± 0.06	2.8 ± 0.2	2.0 ± 0.2	0.43 ± 0.04
	Batch 2	11 ± 1	5.0 ± 0.5	0.32 ± 0.10	0.57 ± 0.08	2.0 ± 0.1	1.5 ± 0.1	0.31 ± 0.03
	Batch 3	12 ± 2	5.9 ± 0.8	1.1 ± 0.2	0.51 ± 0.03	1.6 ± 0.1	0.83 ± 0.10	0.48 ± 0.09
Average value		12 ± 1	5.6 ± 0.6	1.2 ± 0.6	0.57 ± 0.09	2.1 ± 0.8	1.2 ± 0.4	0.62 ± 0.24

3.2. Chickpeas

Raw chickpeas provided a high content of Fe and Zn, whose values ranged between 26 - 76 µg/g for Zn and 34 – 50 µg/g for Fe (Tables 3 – 4). Also, it contained moderate amounts of Mn, with values ranging between 11 – 47 µg/g (Table 6). It has been widely demonstrated that chickpeas are a good source of Fe and Zn (Viadel et al., 2009; Wang et al., 2010; Hemalatha et al., 2007; Thavarajah and Thavarajah, 2012). As with lentils, there were no significant differences in element concentrations studied, expressed as dry matter, between raw and soaked chickpeas (data not shown).

As was the case for soaked lentils, the percentages of bioaccessible minerals found were low for all the elements studied, though being even lower for chickpeas, revealing the high content of antinutritional factors present in this legume (Jukanti et al., 2012; Muzquiz and Wood, 2007; Singh et al., 2015) which can have a considerable impact on its bioaccessibility to the human body. These low percentages of mineral and trace element bioaccessibility in chickpeas have also been identified in previous studies (Singh et al., 2016; Hemalatha et al., 2007; Sahuquillo et al., 2003; Sebastiá et al., 2001). With respect to the solubility assays of soaked chickpeas, the highest percentages of elements corresponded to Cu (22%), followed by Zn (18.6%) and Ca (17.1%). On the other hand, the highest dialyzability percentages were discovered to be Fe and Mg (6.2%), followed by Zn (5.8%), Ca (4.3%) and Cu (3.7%) (see Tables 3 – 8). In general, these results are lower than those reported by other authors (Sahuquillo et al., 2003; Hemalatha et al., 2007; Singh et al., 2016).

Once again, a synergistic effect in chickpeas between Fe and Cu can be observed regarding its bioaccessibility, due to a significant positive interaction between total Cu and soluble Fe ($r = 0.867$; $p<0.05$) and soluble Cu and dialyzable Fe ($r = 0.801$; $p <0.01$). Furthermore, other positive interactions were also observed between soluble Fe - soluble Mg ($r = 0.933$; $p<0.01$) and total Cu - soluble Mg ($r = 0.767$; $p<0.05$). Nevertheless, these later interactions are more difficult to justify.

Like that which occurred with lentils, total mineral content was considerably reduced in cooked chickpeas (see Tables 3 – 8). Thus, in our study, the highest reductions were found in the case of Mn (50.6%) following by Zn (41.1%), Fe (40.9%) and Mg (31.8%), and was minimal in the case of Cu (2.7%). However, this decrease was not observed in the case of Ca. In comparison to the rest of elements studied, an increase in Ca concentration of 5.3% was observed, corresponding to the experience with lentils, which suggests an uptake of this mineral by the legumes from the cooking water.

Regarding the solubility and dializability assays, cooked chickpeas showed higher levels of minerals and trace elements bioaccessible than soaked chickpeas, which could be explained by the positive effect that the heat treatment has on the destruction of antinutritional components (oxalates, phytates, antitrypsins, etc) (Adamidou et al., 2011; Pedrosa et al., 2012; Xu et al., 2016; Shi et al., 2018) and consequent improvement in mineral bioavailability. The highest values were for Fe and Zn both in the solubility test (97.6%; 75.1%) as well as in the dialyzability (45.2%; 24.5%), respectively. Other elements that also showed satisfactory percentages of bioaccessibility were Mn (solubility: 43.6%; dialyzability: 16.8%), and Mg (solubility: 42.7%; dialyzability: 17.3%) (Tables 3 – 8).

As was the case with soaked chickpeas, total Cu concentration influenced soluble Mg concentration (r = 0.672 p < 0.05). Moreover, a negative correlation between total Fe and dialyzable Ca (r = - 0.667; p< 0.05) was found, which highlights once again the negative effect of Fe on the absorption of Ca (Thompson et al., 2010; Lönnerdal, 2010), the same as occurred in the lentil samples. As with lentils, the positive interaction between soluble Ca concentration and soluble Mg concentration (r = 0.901; p < 0.01) in cooked chickpeas can be observed.

Furthermore, chickpeas presented with similar protein values (ranged between 18.3 – 25.2 g/100 g) to the protein content of meat (Genovese & Lajolo, 2001; Pereira and Vicente, 2013) (Table 9). In addition, chickpeas also stood out for having the highest lipid content, which ranged between 2.1 – 4.1 g/100 g - approximately two times higher than other legumes. These values agree with the data presented by other authors (Ratnayake et al., 2001; Yust et al., 2003; de Almeida Costa et al., 2006; Iqbal et al., 2006). In the case of cooked chickpeas, a positive correlation between soluble Mn concentration and protein content (r = 0.726 p < 0.05) was observed. This positive interaction by proteins has already been widely documented for other inorganic elements such as Fe and Zn (Joshi et al., 2014; Bel – Serrat et al., 2014; Cámara-Martos and Moreno-Rojas, 2016; Ramírez – Ojeda et al., 2016).

Table 7
Total and bioaccesible calcium (soluble and dialyzed) analyzed in legumes (µg · g^{-1} fresh matter; mean ± standar desviation).

			Soaked			Cooked		
LENTIL		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	424 ± 23	182 ± 10	113 ± 9	44 ± 4	157 ± 8	90 ± 0	30 ± 2
	Batch 2	459 ± 12	195 ± 5	114 ± 5	45 ± 3	313 ± 20	57 ± 0	29 ± 1
	Batch 3	469 ± 46	220 ± 22	121 ± 3	55 ± 4	154 ± 20	52 ± 0	30 ± 2
Brand 2	Batch 1	479 ± 29	227 ± 14	115 ± 15	16 ± 1	170 ± 9	93 ± 1	16 ± 3
	Batch 2	469 ± 36	242 ± 19	98 ± 22	22 ± 5	165 ± 8	54 ± 17	14 ± 2
	Batch 3	455 ± 26	192 ± 11	50 ± 8	16 ± 2	215 ± 31	64 ± 9	20 ± 2
Brand 3	Batch 1	422 ± 58	204 ± 28	67 ± 7	64 ± 7	269 ± 14	100 ± 12	24 ± 2
	Batch 2	387 ± 44	183 ± 21	61 ± 1	68 ± 5	257 ± 15	51 ± 32	26 ± 5
	Batch 3	490 ± 15	205 ± 6	73 ± 6	54 ± 2	274 ± 12	57 ± 14	27 ± 2
Average value		450 ± 33	206 ± 21	90 ± 27	43 ± 20	219 ± 60	69 ± 20	24 ± 6
CHICKPEA		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	814 ± 71	363 ± 32	153 ± 7.7	63 ± 3	242 ± 20	73 ± 6	52 ± 5
	Batch 2	575 ± 46	241 ± 19	135 ± 2.7	21 ± 1	274 ± 55	125 ± 2	46 ± 3
	Batch 3	735 ± 56	340 ± 26	158 ± 8.9	37 ± 4	291 ± 39	62 ± 10	29 ± 7
Brand 2	Batch 1	675 ± 83	335 ± 42	166 ± 0	33 ± 4	277 ± 24	118 ± 11	24 ± 2
	Batch 2	797 ± 71	397 ± 35	187 ± 41	39 ± 6	378 ± 18	78 ± 2	23 ± 3
	Batch 3	857 ± 150	340 ± 59	95 ± 1	28 ± 5	303 ± 13	73 ± 8	20 ± 2
Brand 3	Batch 1	955 ± 159	452 ± 75	125 ± 37	31 ± 8	301 ± 14	123 ± 5	24 ± 2
	Batch 2	970 ± 93	453 ± 44	132 ± 23	35 ± 5	302 ± 18	146 ± 5	22 ± 4
	Batch 3	1080 ± 141	450 ± 59	125 ± 4	34 ± 5	304 ± 22	83 ± 7	22 ± 3
Average value		828 ± 157	375 ± 71	142 ± 27	36 ± 11	297 ± 37	98 ± 30	29 ± 11
BEAN		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	947 ± 113	421 ± 50	129 ± 2	32 ± 3	333 ± 12	82 ± 4	28 ± 3
	Batch 2	881 ± 67	454 ± 35	106 ± 10	38 ± 4	273 ± 29	68 ± 5	33 ± 2
	Batch 3	1162 ± 285	564 ± 138	75 ± 21	47 ± 4	219 ± 27	22 ± 5	22 ± 1
Brand 2	Batch 1	985 ± 101	469 ± 61	133 ± 19	62 ± 3	313 ± 5	76 ± 17	32 ± 2
	Batch 2	947 ± 131	483 ± 128	12 ± 6	49 ± 3	342 ± 16	75 ± 7	29 ± 5
	Batch 3	960 ± 89	460 ± 45	121 ± 8	61 ± 5	295 ± 16	85 ± 7	33 ± 10
Brand 3	Batch 1	945 ± 82	464 ± 40	135 ± 35	70 ± 7	370 ± 20	85 ± 11	24 ± 1
	Batch 2	963 ± 165	434 ± 74	126 ± 14	66 ± 5	338 ± 17	79 ± 1	21 ± 2
	Batch 3	1005 ± 264	487 ± 128	141 ± 8	67 ± 5	322 ± 48	56 ± 15	23 ± 3
Average value		977 ± 77	471 ± 41	121 ± 20	55 ± 14	312 ± 45	70 ± 20	27 ± 5

Table 8
Total and bioaccesible magnesium (soluble and dialyzed) analyzed in legumes (µg · g-1 fresh matter; mean ± standar desviation).

			Soaked			Cooked		
LENTIL		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	1018 ± 32	437 ± 14	192 ± 25	58 ± 5	51 ± 6	66 ± 2	45 ± 15
	Batch 2	950 ± 26	403 ± 11	213 ± 2	57 ± 7	216 ± 9	53 ± 0	31 ± 3
	Batch 3	906 ± 80	424 ± 37	237 ± 3	68 ± 8	109 ± 5	51 ± 1	34 ± 9
Brand 2	Batch 1	856 ± 10	406 ± 5	220 ± 1	59 ± 1	139 ± 5	88 ± 4	26 ± 2
	Batch 2	929 ± 24	480 ± 12	160 ± 3	65 ± 4	185 ± 14	71 ± 2	29 ± 3
	Batch 3	932 ± 86	393 ± 36	127 ± 1	55 ± 2	222 ± 19	90 ± 4	37 ± 2
Brand 3	Batch 1	808 ± 87	390 ± 42	122 ± 18	87 ± 6	165 ± 12	111 ± 2	33 ± 1
	Batch 2	808 ± 70	382 ± 33	160 ± 0	92 ± 2	142 ± 9	47 ± 35	33 ± 2
	Batch 3	1092 ± 23	457 ± 10	220 ± 3	79 ± 2	162 ± 8	70 ± 2	34 ± 3
Average value		922 ± 94	419 ± 33	184 ± 43	69 ± 14	155 ± 53	72 ± 21	34 ± 5
CHICKPEA		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	1272 ± 46	567 ± 20	260 ± 2	76 ± 8	239 ± 6	97 ± 3	53 ± 6
	Batch 2	1305 ± 93	547 ± 39	232 ± 2	77 ± 5	245 ± 19	139 ± 9	53 ± 9
	Batch 3	1116 ± 47	516 ± 22	241 ± 2	80 ± 12	266 ± 17	85 ± 1	53 ± 15
Brand 2	Batch 1	1197 ± 56	595 ± 28	276 ± 1	72 ± 4	255 ± 12	153 ± 0	43 ± 3
	Batch 2	1139 ± 40	567 ± 20	155 ± 3	75 ± 2	310 ± 7	101 ± 1	44 ± 3
	Batch 3	1138 ± 56	451 ± 22	123 ± 2	60 ± 1	271 ± 0	91 ± 2	41 ± 1
Brand 3	Batch 1	1161 ± 21	550 ± 10	132 ± 4	71 ± 1	313 ± 8	150 ± 6	45 ± 2
	Batch 2	1082 ± 32	505 ± 15	128 ± 4	72 ± 1	262 ± 24	138 ± 5	48 ± 1
	Batch 3	1172 ± 32	489 ± 13	147 ± 0	72 ± 2	296 ± 15	95 ± 1	44 ± 3
Average value		1176 ± 72	532 ± 45	188 ± 62	73 ± 6	273 ± 27	116 ± 28	47 ± 5
BEAN		Total raw	Total soaked	Soluble	Dialyzed	Total cooked	Soluble	Dialyzed
Brand 1	Batch 1	1209 ± 20	537 ± 9	263 ± 0	86 ± 8	275 ± 11	179 ± 4	63 ± 6
	Batch 2	1092 ± 29	563 ± 15	271 ± 16	99 ± 13	226 ± 8	124 ± 4	74 ± 12
	Batch 3	1289 ± 23	626 ± 11	155 ± 6	99 ± 23	201 ± 3	56 ± 0	45 ± 1
Brand 2	Batch 1	1174 ± 29	534 ± 13	273 ± 7	93 ± 2	312 ± 12	145 ± 14	52 ± 2
	Batch 2	1131 ± 28	584 ± 15	265 ± 9	97 ± 3	282 ± 7	84 ± 1	42 ± 1
	Batch 3	1151 ± 21	524 ± 19	266 ± 9	96 ± 3	289 ± 15	118 ± 1	59 ± 2
Brand 3	Batch 1	1194 ± 28	586 ± 14	291 ± 8	104 ± 4	317 ± 11	160 ± 12	49 ± 1
	Batch 2	1129 ± 45	510 ± 20	267 ± 9	95 ± 2	260 ± 10	127 ± 2	38 ± 1
	Batch 3	1121 ± 45	543 ± 22	279 ± 6	95 ± 2	249 ± 12	80 ± 1	40 ± 1
Average value		1166 ± 59	556 ± 37	259 ± 40	96 ± 5	268 ± 38	119 ± 40	51 ± 12

3.3. Beans

As was the case for the other legumes studied, raw beans also stood out for their high total trace element content of Fe and Zn, whose values ranged between 42 – 49 µg/g for Fe and 21 – 37 µg/g for Zn (Tables 3 and 4). These values are in agreement with those reported in previous studies (Viadel et al., 2009; Lazarte et al., 2015). Raw beans were also present Ca concentrations ranging between 881 – 1162 µg/g and Mg concentrations ranging between 1092 – 1289 µg/g (Tables 7 and 8). Similar values for these minerals have also been reported by other authors (Viadel et al., 2009; Campos-Vega et al., 2010; Brigide et al., 2014; Seidu et al., 2015). The lowest values in raw beans were found to be Mn (ranged 11 – 15 µg/g) and Cu (ranged 5.5 – 9.9 µg/g), which is in line with previous studies (Brigide et al., 2014; Akinyele and Shokunbi, 2015) (Tables 5 and 6). As happened for the previous legumes, there were no significant differences in element concentrations studied, expressed as dry matter, between raw and soaked beans (data not shown).

In relation to its bioaccessibility (see Tables 3 – 8), the highest solubility percentages were seen in the case of Cu (25.4%) and Zn (25.2%). Besides these elements, Mg and Fe also showed similar percentages (22.2% and 21.7%, respectively). On the other hand, the lowest values were found to those of Ca (12.4%) and Mn (10.2%). In the case of dialyzability, the tendency is almost repeated. Thus, the elements which showed the highest percentages were Zn (8.8%), Mg (8.2%) and Fe (8.1%), followed by Ca (5.6%) and Mn (4.7%). Alternatively, Cu was the element with the lowest percentage (4.6%). Nevertheless, bioaccessibility was low for all elements studied. This limited bioaccessibility has also been reported by other authors with regard to raw beans (Suliburska and Krejpcio, 2014). There was a positive correlation between soluble Mg concentration and dialyzable Ca concentration ($r = 0.767$; $p<0.05$) in soaked beans.

On the other hand, as was the case with the other legumes discussed above, it was observed that the total mineral content of all the elements studied in the case of cooked beans was lower when compared to soaked, due to minerals from the bean seeds leaching into the water during cooking treatments (Wang et al., 2010; Carvalho et al., 2012). The more significant reduction was observed in the case of Mn with an average reduction of 41.7%, followed by Zn (27.9%), Mg (21.9%) and Cu (13.9%). Nevertheless, the Ca content increased during cooking (10.0%) similar to that occurred with the other legumes described (see Tables 3 – 8).

In relation to the bioaccessibility (solubility and dialyzability assays) of cooked beans, an increase in the percentage of all elements studied was showed with respect to soaked ones, as was described in relation to the previous legumes due to destruction of antinutritional factors (Hemalatha et al., 2007; Ramírez-Cárdenas et al., 2008; Thavarajah et al., 2009; Wang et al., 2010; Shang et al., 2016). Thus, it was found that Fe and Zn were the elements which showed the highest bioaccessible percentages for solubility: 86.1% and 78.7%; and dialyzability: 35.8% and 33.0%, for Fe and Zn respectively (Tables 3 and 4). Once again, as occurred with lentils, a positive interaction between soluble Zn and soluble Fe (r = 0.803; p<0.01) and between dialyzable Zn upon Fe dialyzable (r = 0.944; p< 0.01) was observed in cooked beans. Besides these elements, adequate percentages were found in the case of Mn, which showed a percentage of 56.4% for solubility and 30.0% for dialyzability. Other elements that also showed moderate percentages of bioaccessibility were Cu (38.8% - 25.4%) and Mg (44.5% - 19.1%). Finally, Ca showed the lowest bioaccessibility (22.4% and 8.7% for solubility and dialyzability assays) (see Tables 5 – 8). In relation to the elements' interaction in cooked beans, a positive correlation between soluble Ca and soluble Mg (r = 0.750; p<0.05) and between dialyzable Ca and dialyzable Mg (r = 0.734; p < 0.05) was found. At this point, it must be emphasized that this synergistic effect upon the bioaccessibility between Ca – Mg was observed for three legumes studied, when they are cooked. This effect has also been reported in previous research with complex food matrices (Ramírez – Ojeda et al., 2016; Velasco-Reynold et al., 2010) and it should be studied further more deeply.

Finally, beans stand out for their content of proteins and lipids whose values ranged between 21.4 – 26.7 g/100g and between 1.6 – 3.9 g/100g, respectively, in cooked beans. These values are similar to those demonstrated by other authors (Kutosˇ et al., 2003; Viadel et al., 2009; Brigide et al., 2014; Marquezi et al., 2014).

Table 9
Protein and fat content analyzed in legumes (g · 100 g^{-1} fresh matter; mean ± standar desviation)

		Protein	Fat
LENTIL			
Brand 1	Batch 1	21.70 ± 0.14	2.64 ± 0.12
	Batch 2	22.30± 0.06	1.01 ± 0.05
	Batch 3	20.60 ± 0.01	0.77 ± 0.02
Brand 2	Batch 1	24.40 ± 0.07	1.10 ± 0.06
	Batch 2	29.30 ± 0.09	1.06 ± 0.07
	Batch 3	25.40 ± 0.11	1.62 ± 0.15
Brand 3	Batch 1	26.40 ± 0.03	1.42 ± 0.06
	Batch 2	25.90 ± 0.08	1.34± 0.09
	Batch 3	25.90 ± 0.04	1.22 ± 0.06
Average value		24.60 ± 2.70	1.35 ± 0.54
CHICKPEA			
Brand 1	Batch 1	20.50 ± 0.09	3.44 ± 0.05
	Batch 2	21.50 ± 0.03	4.07 ± 0.19
	Batch 3	22.40 ± 0.02	1.12 ± 0.05
Brand 2	Batch 1	21.20 ± 0.06	3.27 ± 0.14
	Batch 2	20.40 ± 0.01	3.46 ± 0.20
	Batch 3	18.30 ± 0.04	3.13 ± 0.03
Brand 3	Batch 1	25.20 ± 0.02	2.08 ± 0.10
	Batch 2	22.10 ± 0.05	2.20 ± 0.03
	Batch 3	23.40 ± 0.03	2.12 ± 0.20
Average value		21.70 ± 2.00	2.77 ± 0.93
BEAN			
Brand 1	Batch 1	24.80 ± 0.04	1.33 ± 0.02
	Batch 2	23.40 ± 0.06	1.23 ± 0.09
	Batch 3	21.40 ± 0.03	0.86 ± 0.14
Brand 2	Batch 1	24.60 ± 0.05	0.80 ± 0.02
	Batch 2	23.60 ± 0.06	0.69 ± 0.03
	Batch 3	25.60 ± 0.06	0.76 ± 0.08
Brand 3	Batch 1	26.00 ± 0.07	1.01 ± 0.08
	Batch 2	24.90 ± 0.06	1.07 ± 0.11
	Batch 3	26.70 ± 0.04	1.56 ± 0.17
Average value		24.60 ± 1.60	1.03 ± 0.29

3.4. Probabilistic assessment

As it was indicated in the section of material and methods, a probabilistic model was developed to estimate the intake level for minerals and trace elements derived from consumption of 150 - 200 g of legumes in a meal (the study was carried out only with data of the cooked samples or “ready-to-eat”). From the obtained data, the intakes of these inorganic elements contributed by the three legumes analyzed together (lentils, chickpeas or beans) were adjusted to different distributions (gamma, lognormdistribution, betageneral) and distributed in percentiles (Figures 1 and 2). In this way, 50th percentile will guarantee equal and superior contributions for at least 50% of the population.

The models developed from the data of total concentration (Figure 1), showed that legumes can be moderately good dietary sources of Mn, Cu and Fe, with contributions to the DRI (FESNAD, 2010) of 20%, 32% and 20% respectively (a bit less percentage in the case of Fe for female population). It should be noted that only the contributions to the intake provided by the three legumes were determined but the contributions from other ingredients that are usually present in the form of consuming this group of foods (meats or braised meats) have not been considered. On the other hand, the contributions to the DRI of Ca and Mg were low (5 and 12% respectively) showing that cooked legumes are not good dietary sources of these minerals.

In the case of bioaccessible concentration data, was considered only the soluble fraction since it would represent the maximum trace element susceptible to being absorbed in the intestinal lumen. Although the DRI establishment take this aspect into account (not all the micronutrients that we ingest are completely absorbed and used by the organism), the main contributions to the DRI were given for Fe and Cu (15 and 11% respectively), followed by Mn and Zn (8% for both elements) (Figure 2). Once more, the contributions to the DRI of Ca and Mg from bioavailable mineral concentration were low, especially in the case of Ca (1%).

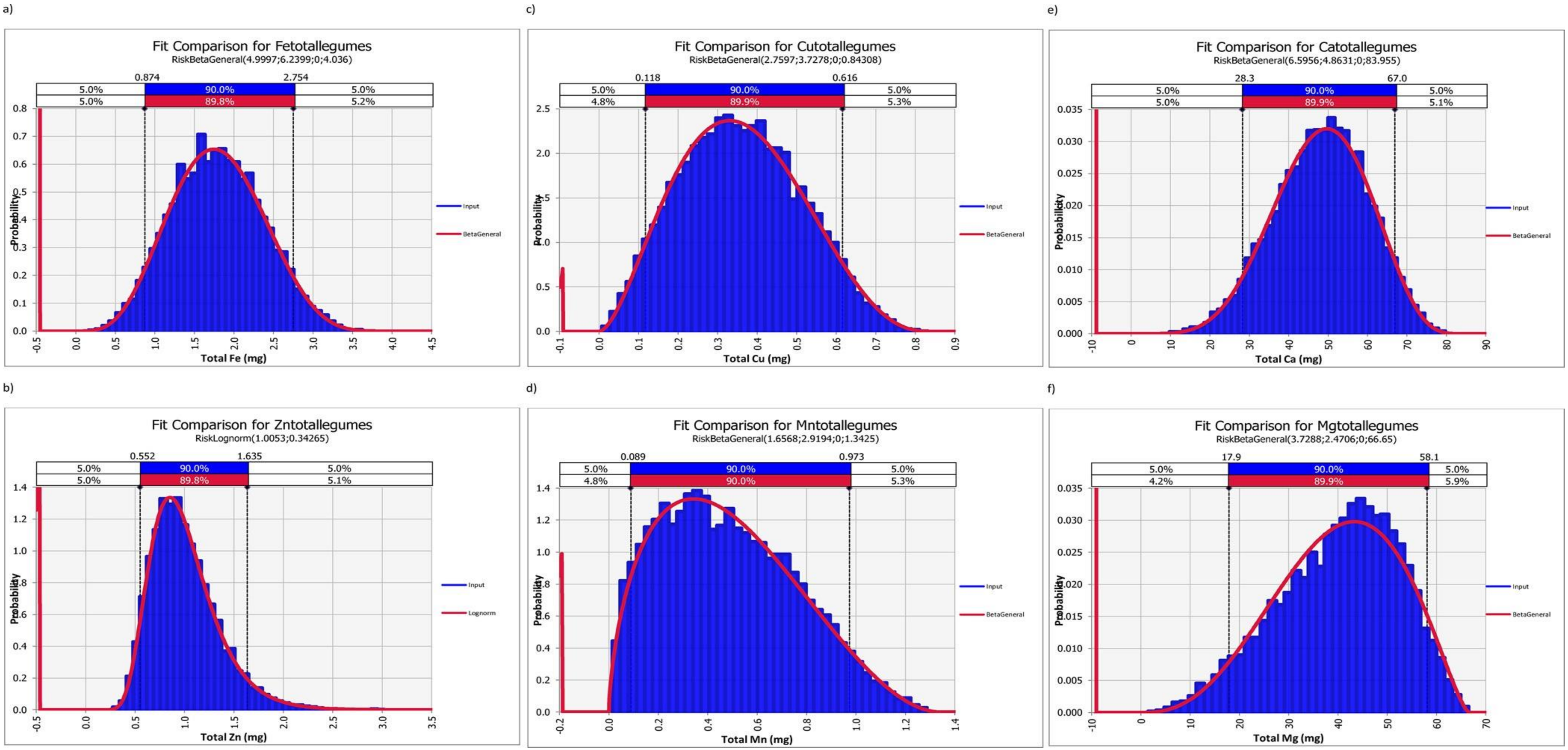

Fig. 1. Simulated data and fitted probabilistic distribution for total trace element content; a) Fe (AIC = - 17087.45; Chi-square = 76.06); b) Zn (AIC = 5294.31; Chi-square = 73.65); c) Cu (AIC = - 9713.15; Chi-square = 105.84); d) Mn (AIC = 110.23; Chi-square = 24.22); e) Ca (AIC = 77652.80; Chi-square = 85.12); f) Mg (AIC = 77760.77; Chi-square = 120.39).

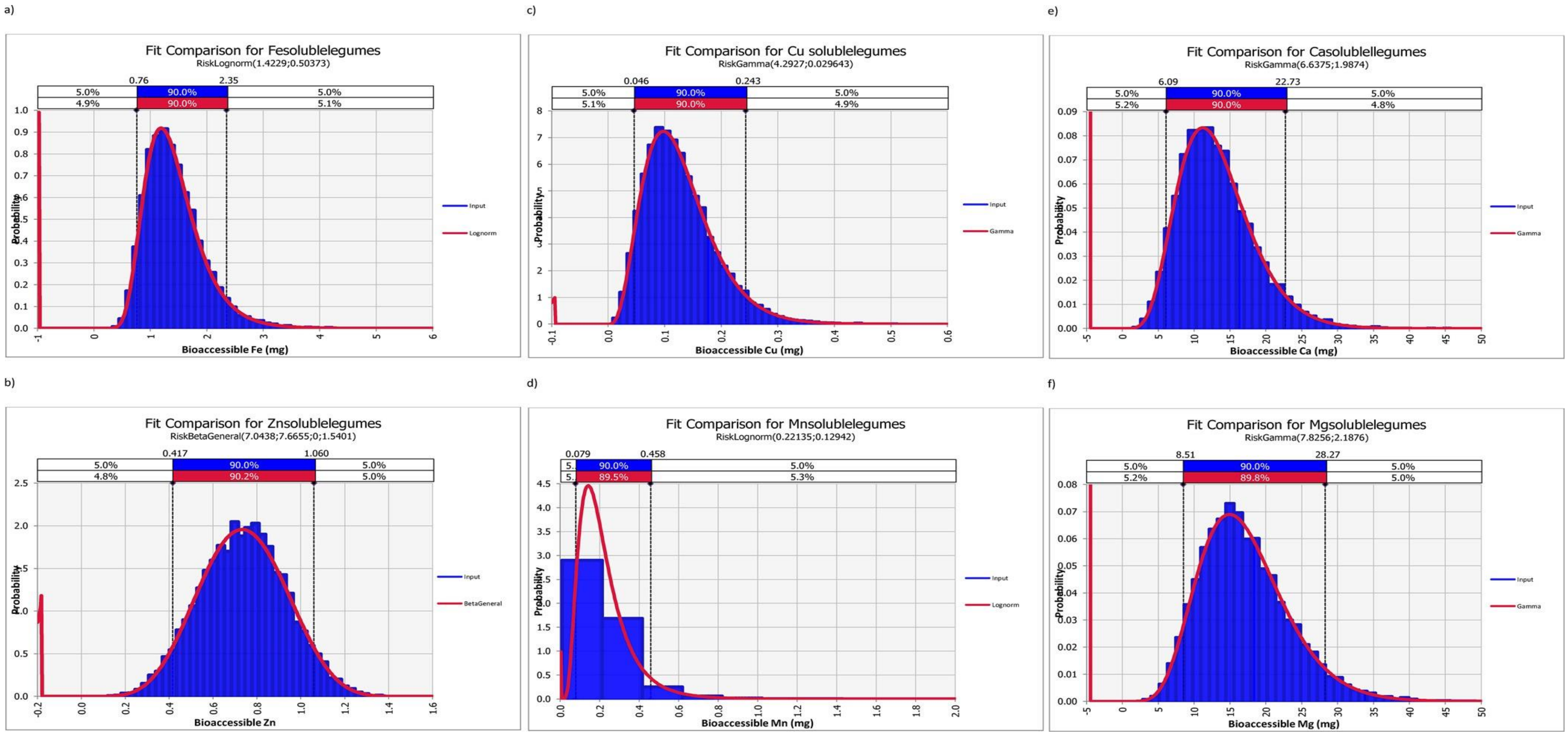

Fig. 2. Simulated data and fitted probabilistic distribution for bioaccessible trace element content; a) Fe (AIC = 12908.99; Chi-square = 84.78); b) Zn (AIC = -4495.58; Chi-square = 88.40); c) Cu (AIC = −29101.14; Chi-square = 59.60); d) Mn (AIC = −1674.84; Chi-square = 38.78); e) Ca (AIC = 60101.41; Chi-square = 62.90); f) Mg (AIC = 63664.06; Chi-square = 82.78).

4. Conclusions

Results showed legumes are distinguishable for their content of Fe and Zn. For all the elements studied (with the noted exception of Ca), the concentration of these micronutrients in the cooked samples was lower than soaked ones, due to a possible migration to these trace elements into the water during cooking treatments. However, the bioaccesibility of these trace elements showed a considered improve in the ready-to-eat sample with respect to the soaked legumes, because of a possible destruction of antinutritionals' components. Among the interactions observed for the different micronutrients present in legumes, it should be highlighted the positive correlation found between the bioaccessibility of Ca-Mg and Fe-Zn, for the legumes studied. It was also observed a negative interaction of Fe upon Ca bioaccessibility as well as another positive correlation between total Cu and solubility Fe. In the case of chickpeas, protein content also contributed to improve the bioaccessibility of Mn.

To conclude, the development of a probabilistic assessment to determine the degree of contribution to the DRI for the elements studied from the consumption of these legumes showed that this group of food can become good or moderate dietary sources of micronutrients such as Fe, Zn, Cu and Mn, so their consumption should be increased, for the sake of a healthier diet, considering also their low fat content and good in proteins of vegetable origin.

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References

Adamidou, S., Nengas, I., Grigorakis, K., Nikolopoulou, D., Jauncey, K., 2011. Chemical composition and antinutritional factors of field peas (*pisum sativum*), chickpeas (*Cicer arietinum*), and faba beans (*Vicia faba*) as affected by extrusion preconditioning and drying temperatures. Cereal Chem. 88, 80–86.

Akinyele, I.O., Shokunbi, O.S., 2015. Concentrations of Mn, Fe, Cu, Zn, Cr, Cd, Pb, Ni in selected Nigerian tubers, legumes and cereals and estimates of the adult daily intakes. Food Chem. 173, 702–708.

Avila, D.S., Gubert, P., Roos, D.H., Puntel, R., Aschner, M., 2016. Manganese. In: In: Caballero, B., Finglas, P.M., Toldrá, F. (Eds.), Encyclopedia of Food and Health 3. Elsevier – Academic Press, Oxford, pp. 637–640.

Bel-Serrat, S., Stammers, A.L., Warthon-Medina, M., Moran, V.H., Iglesia-Altaba, I., Hermoso, M., Moreno, L.A., Lowe, N.M., 2014. Factors that affect zinc bioavailability and losses in adult and elderly populations. Nutr. Rev. 72, 334–352.

Bertinato, J., 2016. Copper: physiology. In: In: Caballero, B., Finglas, P.M., Toldrá, F. (Eds.), Encyclopedia of Food and Health 2. Elsevier – Academic Press, Oxford, pp. 321–326.

Brigide, P., Canniatt-Brazaca, S.G., Silva, M.O., 2014. Nutritional characteristics of bio- fortified common beans. Food Sci. Technol. (Campinas) 34 (3), 493–500.

Cabrera, C., Lloris, F., Gimenez, R., Olalla, M., Lopez, M.C., 2003. Mineral content in legumes and nuts: contribution to the Spanish dietary intake. Sci. Total Environ. 308, 1–14.

Cámara, F., Amaro, M.A., Barberá, R., Clemente, G., 2005. Bioaccessibility of minerals in school meals: comparison between dialysis and solubility methods. Food Chem. 92, 481–489.

Cámara, F., Barberá, R., Amaro, M.A., Farré, R., 2007. Calcium, iron, zinc and copper transport and uptake by Caco-2 cells in school meals: influence of protein and mineral interactions. Food Chem. 100 (3), 1085–1092.

Cámara-Martos, F., Moreno-Rojas, R., 2016. Zinc: properties and determination. In: In: Caballero, B., Finglas, P., Toldrá, F. (Eds.), Encyclopedia of Food and Health 5.
Elsevier – Academic Press, New York, pp. 638–644.

Cámara-Martos, F., Marval-León, J., Moreno-Rojas, R., 2015. In: Morrison, W. (Ed.), Selenium: Dietary Sources, Properties and Role in Human Health, 1st edn. Nova Science Publishers, New York.

Campos-Vega, R., Loarca-Piña, G., Oomah, B.D., 2010. Minor components of pulses and their potential impact on human health. Food Res. Int. 43, 461–482.

Carvalho, L.J., Corrêa, M., Pereira, E., Nutti, M., Carvalho, J.V., Ribeiro, E.G., Freitas, S., 2012. Iron and zinc retention in common beans (Phaseolus vulgaris L.) after home cooking. Food Nutr. Res. 56 156-18.

Chan, W., Brown, J., Lee, S., 1995. Meat, poultry and game. Fifth supplement to McCance and Widdowson's the composition of foods. The Royal Society of Chemistry and the Ministry of Agriculture Fisheries and Food. London.

Chung, C.S., Nagey, D.A., Veillon, C., Patterson, K.Y., Jackson, R.T., Moser-Veillon, P.B., 2002. A single 60-mg iron dose decreases zinc absorption inlactating women. J. Nutr. 132, 1903–1905.

Collins, J.F., Prohaska, J.R., Knutson, M.D., 2010. Metabolic crossroads of iron and copper. Nutr. Rev. 68, 133–147.

De Almeida Costa, G.E., Da Silva Queiroz-Monici, K., Pissini Machado Reis, S.M., De Oliveira, A.C., 2006. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. Food Chem. 94, 327–330.

FAO (2016). www.fao.org/pulses-2016).

FESNAD, 2010. Ingestas Dietéticas de Referencia (IDR) para la población española.
Actividad Dietética 14 (4), 196–197.

Genovese, M.I., Lajolo, F.M., 2001. Atividade inibitória de tripsina do feijão (Phaseolus vulgaris

L.): avaliação crítica dos métodos de determinação. Archivos Latinoamericanos de Nutrição 51, 386–394.
Gulec, S., Collins, J.F., 2014. Molecular mediators governing iron-copper interactions. Annu. Rev. Nutr. 34, 95–116.
Ha, J.H., Doguer, C., Wang, X., Flores, S.R., Collins, J.F., 2016. High-iron consumption impairs growth and causes copper-deficiency anemia in weanling Sprague-Dawley rats. PLoS One 11, 1–19.
Harris, E.D., 2001. Copper and iron: a landmark connection of two essential metals. J. Trace Elem. Exp. Med. 14, 207–210.
Hefnawy, T.H., 2011. Effect of processing methods on nutritional composition and anti-nutritional factors in lentils (Lens culinaris). Ann. Agric. Sci. 56, 57–61.
Hemalatha, S., Platel, K., Srinivasan, K., 2007. Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India. Food Chem. 102 (4), 1328–1336.
Hemalatha, S., Gautam, S., Platel, K., Srinivasan, K., 2009. Influence of exogenous iron, calcium, protein and common salt on the bioaccessibility of zinc from cereals and legumes. J. Trace Elem. Med Biol. 23, 75–83.
Iqbal, A., Khalil, I.A., Ateeq, N., Sayyar Khan, M., 2006. Nutritional quality of important food legumes. Food Chem. 97, 331–335.
Iyengar, V., Pullakhandam, R., Nair, K.M., 2012. Coordinate expression and localization of iron and zinc transporters explain iron-zinc interactions during uptake in Caco-2 cells: implications for iron uptake at the enterocyte. J. Nutr. Biochem. 23, 1146–1154.
Jezierny, D., Mosenthin, R., Bauer, E., 2010. The use of grain legumes as A protein source in pig nutrition: a review. Anim. Feed Sci. Technol. 157, 111–128.
Jodral-Segado, A.M., Navarro-Alarcón, M., 2003. Magnesium and calcium contents in foods from SE Spain: influencing factors and estimation of daily dietary intakes. Sci. Total Environ. 312, 47–58.
Johnson, C.R., Thavarajah, D., Thavarajah, P., 2013. The influence of phenolic and phytic acid food matrix factors on iron bioavailability potential in 10 commercial lentil genotypes (Lens culinaris L.). J. Food Compos. Anal. 31, 82–86.
Joshi, V., Thatte, P., Prakash, J., Jyothi Lakshmi, A., 2014. Effect of oilseed protein concentrates and exogenous amino acids on the dialysability of iron and zinc. LWT - Food Sci. Technol. 59, 540–546.
Jukanti, A.K., Gaur, P.M., Gowda, C.L.L., Chibbar, R.N., 2012. Nutritional quality and health benefits of chickpea (Cicer arietinum L.): A review. Br. J. Nutr. 108, S11–S26.
Khattab, R.Y., Arntfield, S.D., 2009. Nutritional quality of legume seeds as affected by some physical treatments. Antinutritional factors. LWT - Food Sci. Technol. 42, 1113–1118.
Kutosˇ, T., Golob, T., Kacˇ, M., Plestenjak, A., 2003. Dietary fibre content of dry and processed beans. Food Chem. 80 (2), 231–235.
Lazarte, C.E., Carlsson, N.G., Almgren, A., Sandberg, A.S., Granfeldt, Y., 2015. Phytate, zinc, iron and calcium content of common Bolivian food, and implications for mineral bioavailability. J. Food Compos. Anal. 39, 111–119.
Lombardi-Boccia, G., Aguzzi, A., Cappelloni, M., Di Lullo, G., Lucarino, M., 2003. Total- diet study: dietary intakes of macro elements and trace elements in Italy. Br. J. Nutr. 90, 1117–1121.
Lonnerdal, B., 2010. Calcium and iron absorption - mechanisms and public health relevance. Int. J. Vitam. Nutr. Res. 80, 293–299.
Máñez, G., Alegría, A., Farré, R., Frígola, A., 2002. Effect of traditional, microwave and industrial cooking on inositol phosphate content in beans, chickpeas and lentils. Int. J. Food Sci. Nutr. 53, 503–508.
Marquezi, M., Gervin, V.M., Watanabe, L.B., Moresco, R., Amante, E.R., 2017. Chemical and functional properties of different common Brazilian bean (Phaseolus vulgaris L.) Cultivars. Braz. J. Food Technol. 20.

Mazur, A., Maier, J.A.M., 2016. Magnesium. Encyclopedia of Food and Health 3. pp. 587–592.

Mercasa (2015) Alimentación en España 2015. Legumbres, 89–99. http://www.mercasa-ediciones.es/alimentacion_2015/pdfs/ /pag_091-101_legumbres.pdf).

Mohan, V.R., Tresina, P.S., Daffodil, V.O., 2016. Antinutritional factors in legume sedes: characteristics and determination. In: Caballero, B., Finglas, P.M., Toldrá, F. (Eds.), Encyclopedia of Food and Health. Elsevier: Academic Press, Oxford, pp. 211–220.

Moreno-Rojas, R., Amaro-López, M.A., Zurera-Cosano, G., 1994. Copper, iron and zinc variations in Manchego-type cheese during the traditional cheese-making process. Food Chem. 49, 67–72.

Muzquiz, M., Wood, J.A., 2007. Antinutritional factors. In: Yadav, S.S., Redden, B., Chen, W., Sharma, B. (Eds.), Chickpea Breeding and Management. CAB International, Wallingford, pp. 143–166.

O'Brien, K.O., Zavaleta, N., Caulfield, L.E., Wen, J., Abrams, S.A., 2000. Prenatal iron supplements impair zinc absorption in pregnant Peruvian women. J. Nutr. 130, 2251–2255.

Olmedilla Alonso, B., Farré Rovir, R., Asensio Vegas, C., Martín Pedrosa, M., 2010. Papel de las leguminosas en la alimentación actual. Actividad Dietética 14, 72–76.

Ortega, R.M., López-Sobaler, A.M., Requejo, A.M., Andrés, P., 2004. La Composición de los Alimentos. Herramienta básica para la valoración nutricional, 1st ed. Editorial Complutense, Madrid.

Pedrosa, M.M., Cuadrado, C., Burbano, C., Allaf, K., Haddad, J., Gelencsér, E., Takács, K., Guillamón, E., Muzquiz, M., 2012. Effect of instant controlled pressure drop on the oligosaccharides, inositol phosphates, trypsin inhibitors and lectins contents of different legumes. Food Chem. 131, 862–868.

Pereira, P.M., Vicente, A.F., 2013. Meat nutritional composition and nutritive role in the human diet. Meat Sci. 93, 586–592.

Ramírez-Cárdenas, L., Leonel, A.J., Costa, N.M.B., 2008. Efeito do processamento doméstico sobre o teor de nutrientes e de fatores antinutricionais de diferentes cultivares de feijão comum. Ciência e Tecnologia Alimentaria 28, 200–213.

Ramírez-Cárdenas, L., Leonel, A.J., Costa, N.M.B., Reis, F.P., 2010. Zinc bioavailability in different beans as affected by cultivar type and cooking conditions. Food Res. Int. 43, 573–581.

Ramírez-Ojeda, A.M., Moreno, –, Rojas, R., Sevillano, –, Morales, J., Cámara, –, Martos, F., 2016. Influence of dietary components on minerals and trace elements bioaccessible fraction in organic weaning food: a probabilistic assessment. Eur. Food Res. Technol. 243 (4), 639–650.

Ratnayake, W.S., Hoover, R., Shahid, F., Perera, C., Jane, J., 2001. Composition, molecular structure, and physicochemical properties of starchs from four field peas (Pisum sativum) L. cultivars. Food Chem. 74, 189–202.

Sahuquillo, A., Barberá, R., Farré, R., 2003. Bioaccessibility of calcium, iron and zinc from three legume samples. Nahrung Food 47, 438–441.

Sebastiá, V., Barberá, R., Farré, R., Lagarda, M.J., 2001. Effects of legume processing on calcium, iron and zinc contents and dialysabilities. J. Sci. Food Agric. 81, 1180–1185.

Seidu, K.T., Osundahunsi, O.F., Olaleye, M.T., Oluwalana, I.B., 2015. Amino acid com- position, mineral contents and protein solubility of some lima bean (Phaseolus lunatus l. Walp) seeds coat. Food Res. Int. 73, 130–134.

Shang, R., Wu, H., Guo, R., Liu, Q., Pan, L., Li, J., Hu, Z., Chen, C., 2016. The diversity of Four anti-nutritional factors in Common bean. Hortic. Plant J. 2, 97–104.

Shi, L., Arntfield, S.D., Nickerson, 2018. Changes in levels of phytic acid, lectins and oxalates during soaking and cooking of Canadian pulses. Food Res. Int. 107, 660–668.

Singh, P.K., Shrivastava, N., Sharma, B., Bhagyawant, S.S., 2015. Effect of domestic processes on chickpea seeds for antinutritional contents and their divergence. Am. J. Food Sci.

Technol. 3, 111–117.

Singh, P., Prasad, S., Aalbersberg, W., 2016. Bioavailability of Fe and Zn in selected legumes, cereals, meat and milk products consumed in Fiji. Food Chem. 207, 125–131.

Suliburska, J., Krejpcio, Z., 2014. Evaluation of the content and bioaccessibility of iron, zinc, calcium and magnesium from groats, rice, leguminous grains and nuts. J. Food Sci. Technol. 51, 589–594.

Tharanathan, R.N., Mahadevamma, S., 2003. Grain legumes – a boon to human nutrition. Trends Sci. Technol. 14 (12), 507–518.

Thavarajah, D., Thavarajah, P., 2012. Evaluation of chickpea (Cicer arietinum L.) micronutrient composition: Biofortification opportunities to combat global micro- nutrient malnutrition. Food Res. Int. 49, 99–104.

Thavarajah, P., Thavarajah, D., Vandenberg, A., 2009. Low phytic acid lentils (Lens culinaris L.): A potential solution for increased micronutrient bioavailability. J. Agric. Food Chem. 57, 9044–9049.

Thavarajah, D., Thavarajah, P., See, C.T., Vandenberg, A., 2010. Phytic acid and Fe and Zn concentration in lentil (Lens culinaris L.) seeds is influenced by temperature during seed filling period. Food Chem. 122, 254–259.

Thompson, B.A., Sharp, P.A., Elliot, R., Fairweather, –, Tait, S.J., 2010. Inhibitory effect of calcium on non-heme iron absorption may be related to translocation of DMT-1 at the apical membrane of enterocytes. J. Agric. Food Chem. 58, 8414–8417.

Torija, M.E., Díez, C., 1999. Legumbres. En: Tratado de nutrición. In: Hernández, M., Sastre, A. (Eds.), Ediciones Díaz de Santos, pp. 425–429 Madrid, España.

Troost, F.J., Brummer, R.J., Dainty, J.R., Hoogewerff, J.A., Bull, V.J., Saris, W.H., 2003. Iron supplements inhibit zinc but not copper absorption in vivo in ileostomysubjects. Am. J. Clin. Nutr. 78, 1018–1023.

Vashishth, A., Ram, S., Beniwal, V., 2017. Cereal phytases and their importance in im-provement of micronutrients bioavailability. 3 Biotech 7, 42.

Velasco-Reynold, C., Navarro-Alarcón, M., López-Gª de la Serrana, H., Pérez-Valero, V., Agil, A., López-Martínez, M.C., 2010. Dialysability of magnesium and calcium from hospital meals: influence exerted by other elements. Biol. Trace Elem. Res. 133, 313–324.

Viadel, B., Barberá, R., Farré, R., 2006a. Effect of cooking and legumes species upon calcium, iron and zinc uptake by Caco-2 cells. J. Trace Elem. Med Biol. 20, 115–120.

Viadel, B., Barberá, R., Farré, R., 2006b. Uptake and retention of calcium, iron, and zinc from raw legumes and the effect of cooking on lentils in Caco-2 cells. Nutr. Res. 26, 591–596.

Viadel, B., Barberá, R., Farré, R., 2009. Calcium, iron and zinc uptakes by Caco-2 cells from white beans and effect of cooking. Int. J. Food Sci. Nutr. 57 (3-4), 190–197.

Wang, N., Daun, J.K., 2006. Effects of variety and crude protein content on nutrients and anti-nutrients in lentils (Lens culinaris). Food Chem. 95, 493–502.

Wang, N., Hatcher, D.W., Toews, R., Gawalko, E.J., 2009. Influence of cooking and dehulling on nutritional composition of several varieties of lentils (Lens culinaris). LWT - Food Sci. Technol. 42, 842–848.

Wang, N., Hatcher, D.W., Tyler, R.T., Toews, R., Gawalko, E.J., 2010. Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). Food Res. Int. 43 (2), 589–594.

Xu, Y., Cartier, A., Obielodan, M., Jordan, K., Hairston, T., Shannon, A., Sismour, E., 2016. Nutritional and anti-nutritional composition, and in vitro protein digestibility of Kabuli chickpea (*Cicer arietinum* L.) as affected by differential processing methods. J. Food Meas. Charact. 10, 625–633.

Yamaji, S., Tennant, J., Tandy, S., Williams, M., Singh Srai, S.K., Sharp, P., 2001. Zinc regulates the function and expression of the iron transporters DMT1 and IREG1 in human intestinal Caco-2 cells. FEBS Lett. 507, 137–141.

Yust, M.M., Pedroche, J., Giron-Calle, J., 2003. Production of ACE inhibitory peptides by digestion of chickpea legumin with alcalase. J. Food Chem. 81, 363–369.


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3.3. CAPÍTULO 3: Influence of dietary components on minerals and trace elements bioaccessible fraction in organic weaning food: a probabilistic assessment

A. M. Ramirez-Ojeda[1] · R. Moreno-Rojas[1] · J. Sevillano-Morales[1] · F. Camara-Martos[1]

1 Departamento de Bromatología y Tecnología de los Alimentos, Campus Universitario de Rabanales, Edificio Charles Darwin C-1, 14014, Córdoba, Spain

Author for correspondence: Prof. Fernando Cámara-Martos, PhD.

Tel.: + 34 957 212 026

Fax: + 34 957 212 000

e-mail: bt2camaf@uco.es

Abstract The Fe, Zn, Mn, Cu, Ca and Mg contents as well as bioaccessible fractions of ten weaning foods characterized and commercialized with the attribute "organic" were analyzed in order to evaluate them nutritionally. The influence of several dietary components on minerals and trace elements bioaccessibility was also studied. It was observed a positive correlation ($p < 0.05$; $r = 0.830$) between protein content and Fe solubility for all samples analyzed with the exception of a jar. According with data supplied by manufacturer for this jar, the high solubility of Fe, despite the low protein content, could be due to the presence of vitamin C as ingredient. The influence of proteins was also observed in the dialysability of Zn ($p < 0.01$; $r = 0.998$) for the weaning food that incorporated meat or legumes (chickpeas) in their list of ingredients. On the other hand, the amount of Mn dialyzable was higher when a lower amount of fat was present in the jar formulation ($p < 0.01$; $r = - 0.781$). Several interactions between trace elements were also observed, highlighting a positive soluble Cu–dialyzable Fe interaction ($p < 0.01$; $r = 0.818$). Trace elements concentration and bioaccessible values obtained were considerably lower than those reported for weaning food without the attribute organic. It has also developed estimation to the daily intake of these elements using a probabilistic approach. The contribution of Fe, Zn and Ca to the dietary reference intakes for jars studied was below 2.5 and 5 % considering soluble and total content values, respectively, for 50 % population.

Keywords Trace elements · Weaning food · Bioaccessibility · Probabilistic assessment · Organic

Introduction

The conventional farming techniques using fertilizers and inorganic pesticides that can produce both environmental and public health problems in the long term as they increase the content of contaminants in plants and soil [1]. Although pesticide exposure can occur through different routes (inhalation, dermal exposure), food has been recognized as the main exposure route to pesticide residues for consumers not working with them [2]. Several studies have shown that pesticide residues to several levels have been detected in a range of 63–52 % of fruit and vegetable samples [3, 4]. Besides, heavy metal contamination of vegetable foods may be occurred due to irrigation with contaminated water and/or the addition of metal-based pesticides [5] and through feed intake, is further transferred to animals [6].

Organic farming constitutes an alternative; indeed, it is progressing in recent years as an option to integrate new agricultural techniques oriented to address this growing consumer concern about health and food safety. The term “organic food” refers to food that is free from artificial compounds such as chemical fertilizers and pesticides, veterinary drugs, hormones and antibiotics, and genetically modified organisms [7].

This tendency on the part of some sectors of the population to consume organic food has been also translated to the case of baby foods even though the research data on the nutritional benefits of organic food compared to their non-organic homologues are scarce and contradictory [8, 9]. However, the nutritional composition of these baby foods should be monitored very strictly as they must present a highly nutritional protein, energy and micronutrients such as vitamins and trace elements. Previous studies have shown that optimal nutrition during critical periods in life, such as early infancy, ensures long-term consequences on late physiological and metabolic processes and may play a role in the reduction of onset of diseases [10].

In the literature, there are several studies assessing the content of minerals and trace elements necessary for proper development, present in infant formulas or processed cereal-based food [11–18], but studies on weaning foods are scarce [19–21]. This is important if considering that the infants start to introduce these foods (6–12 months) in the period where major changes occur in both macronutrients and micronutrients intakes [22]. Furthermore, only determining the content of trace elements present in a weaning food may not be sufficient to evaluate its nutritional quality. We should note that only a fraction of the concentration initially present in the feed will be efficiently absorbed, metabolized by typical routes and used by the body for typical physiological functions or deposited in storage compounds, thus resulting in the concept of bioavailability [23].

A first step in the evaluation of mineral and trace element bioavailability is to determine the bioaccessible fraction, which can be obtained by subjecting the food to a process of gastrointestinal digestion in vitro similar to that occurring in the human gastrointestinal tract. Moreover, these bioaccessibility assays allow us to distinguish between soluble fraction (that remaining soluble in the lumen and thus it is capable of being absorbed) and dialyzable fraction (which besides solubilized, it is capable of crossing a membrane with a pore size) [24, 25]. Besides, the bioaccessibility assays also allow us to easily determine the influence of other nutritional components (proteins, fat, vitamins, and other trace elements) in the capacity of a trace element to be absorbed [26].

With all this, the aim of this study was to determine the content and bioaccessibility (solubility and dialysability) of minerals and trace elements present in weaning food categorized with the "organic" attribute, in order to evaluate nutritionally. It has also developed an estimation to the daily intake of these elements using a probabilistic approach. Likewise, the influence of certain food components (dietary factors) in the bioaccessibility of these minerals and trace elements is also studied. Obtaining these data allow us to make a correct formulation with the ingredients of these weaning foods, thus improving the bioaccessibility of trace elements initially present.

Materials and methods

Materials and reagents

All reagents were of analytical-reagent grade. Ultrapure water (18 MΩ/SCF) prepared with a Milli-Q Reference Water Purification (Millipore, Madrid, Spain) was used throughout experiments. All glassware and plastic containers were soaked in 10 % nitric acid overnight and rinsed three times with de-ionized water prior to use. Nitric acid (65 %) and hydrochloric acid (35 %) was obtained from Panreac (Barcelona, Spain). Lanthanum chloride was supplied by Perkin Elmer (Madrid, Spain). Sodium bicarbonate (97 %) was obtained from Scharlau (Barcelona, Spain).

Digestive enzymes and bile salts were supplied by Sigma-Aldrich Co. (St. Louis, MO). The working solutions of these enzymes were prepared immediately before use. Pepsin solution was obtained by dissolving 3.2 g of pepsin (P-7000 from porcine gastric mucosa) in 20 mL of

HCl (0.1M). The solution of pancreatin and bile salts were prepared by dissolving 0.6 g of pancreatin (P-3292 from porcine pancreas) and 3.9 g of bile salts (B-8756 of porcine origin) in 150 mL of 0.1M $NaHCO_3$. The dialysis membranes, with a pore size (MWCO) of 12-14,000 Å (Size 6 Inf Dia 27/32”-21.5 mm, 30 m, Bestlno. 1063F09, Medicell Int. LTD, London, UK), were rinsed several times with distilled deionized water before use.

Standard solutions for measuring the elements Fe, Zn, Cu, Mn, Ca and Mg, and were prepared immediately before use by dilution with distilled deionized water of a 1000 mg L^{-1} standard solutions (Scharlau Chemie, Barcelona, Spain).

Samples

The Fe, Zn, Cu, Mn, Ca and Mg contents were analyzed in 10 commercialized weaning foods characterized with the attribute “organic” (jars of 200 g). These weaning foods were received at the University of Cordoba (Spain) proceeding from a factory specialized on the manufacture and marketing of organic food. Upon arrival at the laboratory, samples were poured in plates, freezed—dried and packed in polypropylene vacuum bags, until required for analyses.

All ingredients used in the formulation of different weaning food are described in Table 1. These weaning foods are recommended for infants from 1 year old. They can make up a unique type of food or to be consumed occasionally at older ages (3–4 years). For mineral content determination, a total of 50 samples were analyzed, corresponding to one sample taken from five different jars for each type of weaning food. In order to get a representative sample, this procedure was repeated for the bioaccessibility assays (solubility and dialysability). The total samples analyzed in the three determinations (mineral content, solubility and dialysability assays) were 150.

Table 1 Ingredients used in the formulation of weaning food analyzed (data supplied by manufacturer)

	Code	Ingredients
Banana, pear and pomegranate	BPP	Fruit 97.5% (banana, pear, pomegranate juice, lemon juice), flour 2.5%, vitamin C
Vegetables, chickpeas and pear	VCP	Cooking water and vegetables 45.5% (potato, squash, tomato, onion, green beans), chickpeas 6%, pear 3%, flour 2.5%, extra virgin olive oil 2%, paprika spices 0.14%
Vegetables, veal	VV	Vegetables 67.5% (tomato, potato, onion, peas, carrot), veal 12%, cooking water, flour 2.5%, extra virgin olive oil and spices 2%
Banana, apple, orange	BAO	Fruit 100% (banana, apple, orange juice, lemon juice), vitamin C
Multifruit	MFR	Fruit 95.5% (banana, apple, orange juice, for, grape juice, lemon juice), carrot juice 4.5%, vitamin C
Zucchini creme	ZC	Vegetables 75% (zucchini, potato, leek, onions), cooking water, extra virgin olive oil
Squash, chamomole flower	SCF	Cooking water and vegetables 69.5% (squash, potato, onion, leek), extra virgin olive oil 2%, peppermint and chamomile flower
Varied vegetables creme	VVC	Cooking water and vegetables 72% (potato, leek, green beans, onion, zucchini) and extra virgin olive oil 2%
Vegetables, chicken	VC	Cooking water and vegetables 56% (potato, tomato, onion, red pepper), chicken 12%, extra virgin olive oil 2% parsley and spices
Vegetables, chicken, pasta	PVC	Cooking water and vegetables 51% (potato, tomato, onion), chicken 12%, wholemeal pasta 4%, extra virgin olive oil 2% and spices

Procedure for in vitro gastrointestinal digestion

Solubility assay

The procedure described by Cámara et al. [25] with slight modifications was used to estimate soluble trace element solubility. The simplified process is based on mimicking the physiological conditions of the gastrointestinal tract, i.e., chemical composition of digestive fluid, pH and typical residence time for each step of the digestion process. Thus, 3 g of lyophilized sample of weaning food were homogenized with 22 mL of 0.1N HCl. To acidify the pH was adjusted to 2 with 6N HCl.

To carry out pepsin–HCl digestion, 0.5 g of pepsin solution per 100 g of homogenized was added (corresponding to 0.125 g of porcine pepsin by 3 g of lyophilized sample). The mixture was then incubated for 2 h at 37 °C in a shaking water bath (HSB-2000 Shaking Bath; E-Chrom Tech CO., LTD, Taipei, Taiwan). After this time, to stop gastric digestion, the sample was maintained for 10 min in an ice bath.

Then, the pH was adjusted to 5 by adding 1M $NaHCO_3$ to continue with intestinal digestion step. Then, 6.2 mL of a mixture of pancreatin and bile salts (corresponding to 0.025 g of pancreatin and 0.160 g of bile salts by 3 g of lyophilized sample) was added to each test tube, which were incubated for 2 h more. After intestinal digestion, tubes are submerged for 10 min in an ice bath to stop the action of this mixture of enzymes.

Finally, the pH was adjusted to 7.2 with 0.5M NaOH. Aliquots of the digested sample were transferred to polypropylene centrifuge tubes (50 mL, Costar Corning Europe, Badhoevedorp, the Netherlands), and these were centrifuged for 1 h at 4000 rpm and 4 °C (Eppendorf Centrifuge 5810 R). Then, the supernatant (soluble fraction) was collected, its organic matter was destroyed, and the mineral content was measured by atomic absorption spectrometry.

Dialysability assay

For the measurement of the dialyzable fraction, we started from 5 g of lyophilized sample. A procedure similar to solubility assay was followed to the stage of gastric digestion with pepsin. Prior to the intestinal digestion step a dialysis bag (molecular mass cut-off 12–14,000 Å) containing 25 mL of deionized water with an amount of $NaHCO_3$ equivalent to the titratable acidity previously measured [25] was placed in the flasks. Incubation was continued for 45 min, the pancreatic–bile salt mixture was added, and incubation was continued up to 2 h. After incubation, the dialysis membranes were removed from the flasks, rinsed with deionized water. The dialyzable fraction was transferred to porcelain crucibles, its organic matter was destroyed, and the mineral content was measured by atomic absorption spectrometry.

Trace element determination

To determine total trace element content of weaning foods, 1 g of lyophilized sample was weighed in a porcelain crucible. The samples were incinerated in a muffle furnace at 460 °C for 15 h. The ash was bleached after cooling by adding 2.5 mL of 2N HNO_3, drying on thermostatic hotplates, and maintaining in a muffle furnace at 460 °C for 1 h more. Ash recovery was performed with 1 mL of HCl 6N, making up to 10 mL with deionized water.

To determine trace element content in soluble and dialyzable fraction obtained in 2.3 a similar procedure was followed.

Elemental analyses were performed by flame absorption atomic spectroscopy (FAAS) with a Varian Spectra AA—50B model, equipped with standard air-acetylene flame, and single element hollow cathode lamps. Electrothermal atomic absorption spectrometry (ETAAS) was used for the determination of Cu and Mn in soluble and dialyzable fraction by a Perkin-Elmer model Analyst 600 with graphite furnace and an autosampler. The instrumental conditions for the determination are shown in Tables 2 and 3. The detection limit was calculated as the mean value of 30 measurements of the blank plus three times their standard deviation. Regarding the quantification limit, it was calculated as the mean value of 30 measurements of the blanks plus 10 times their standard deviation.

AOAC methods were used to determine the proximate composition: defatting in a Soxhlet apparatus with petroleum ether for crude fat and micro-Kjeldahl for protein. Briefly, to Kjeldahl method, 0.5 g of lyophilized sample with a catalyst pellet was placed in digestion flask and 20 mL of concentrated sulfuric acid was added. The mixture was heated in a digester until solution clears. After cooling with 70 mL of deionized water, the solution was distilled with a small quantity of sodium hydroxide, which converts the ammonium salt to ammonia. The ammonia gas is led into a trapping solution of hydrochloric acid 0.1N and determined by back titration with sodium hydroxide solution 0.1N. The protein concentration was calculated from the nitrogen values using a conversion factor of 6.25. For the determination of fat content, 3–5 g of lyophilized sample were weighed and wrapped in a paper filter and subsequently transferred into a Soxhlet liquid/solid extractor with petroleum ether during 1 h. After fat extraction, samples were dried in a desiccators and weighed.

Table 2
Instrumental conditions, limit of detection, limit of quantification and analysis of certified references materials.

Element	Wavelength (nm)	Slit Width (nm)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Certified references material (mg kg^{-1})					
					Rice flour NIST – 1568a			Bovine liver BCR – 185R		
					Certified	Found	Recovery (%)	Certified	Found	Recovery (%)
Fe	248.3	0.2	0.084	0.28	7.42 ± 0.44	7.58 ± 0.52	102	-	-	
Zn	213.9	0.7	0.168	0.56	19.42 ± 0.26	20.38 ± 0.24	105	138.6 ± 2.1	130.4 ± 17.1	94
Mn	279.5	0.2	0.013	0.043	19.20 ± 1.80	18.48 ± 4.20	96	11.07 ± 0.29	11.32 ± 2.87	102
Cu	324.8	0.7	0.014	0.05	2.35 ± 0.16	2.26 ± 0.34	96	277 ± 5	264 ± 38	95
Ca	422.7	0.7	0.315	1.05	118.4 ± 3.1	116.7 ± 2.1	98	-	-	
Mg	285.2	0.7	0.011	0.036	559 ± 10	556 ± 18	99	-	-	

LOD limit of detection, *LOQ* limit of quantification

Table 3 Instrumental operating parameters for Cu and Mn ET – AAS determination

Step	*T* (°C)	Ramp time (s)	Hold time (s)	Internal flow (mL min^{-1})
Drying	110	15	20	250
Pyrolysis	900	10	20	250
Atomization	2000	0	4	0
Cleaning	2600	1	3	250

Statistics and probabilistic assessment

Data were analyzed using SPSS 15.0 (IBM, Armonk, NY). Pearson's correlation coefficient (parametric conditions) and Spearman's correlation coefficient (nonparametric conditions) were used for determining dependence between variables. Significant differences were considered when $p < 0.05$.

A probabilistic model was developed to estimate the intake level for Fe, Zn, Ca, Mg, Mn and Cu derived from consumption of weaning foods. The model here developed followed a probabilistic approach in which variables were described by probability distributions.

Probability distributions were fitted to concentration data obtained in our study for each element. Furthermore, in order to estimate the intake level, serving size was considered. However, since no data were found concerning the consumption patterns of this type of product, we assumed a serving size ranging 150–200 g for infants between 1 and 3 years which was defined by a uniform distribution in the probabilistic model which means that all values in that range had the same probability to occur.

The exposure model did not include a separation between the variability and uncertainty of input variables (so-called first order model). The goodness of fitting to data was assessed by using different statistical tests such as which corresponded to Kolmogorov–Smirnov test and Chi-square test. These statistical tests allow to give a guess of how well the fitted distribution described the observed data. In addition, the visual analysis was equally considered to assess the fitting of the probability distributions to concentration data. The model was simulated using a simulation algorithm implemented in Excel Microsoft © software. The simulation was run using 10,000 iterations for each element and the value of the seed used for the Random Number Generator on which the simulation was based to single value in all simulation carried out in order to make results comparable.

Results and discussion

Table 4. Content of Fe, Zn, Mn, Cu in the weaning food studied.

		Fe			Zn			Mn			Cu		
Sample	Humidity (%)	Total (μg g^{-1})	Soluble (μg g^{-1})	Dialyzable (μg g^{-1})	Total (μg g^{-1})	Soluble (μg g^{-1})	Dialyzable (μg g^{-1})	Total (μg g^{-1})	Soluble (μg g^{-1})	Dialyzable (μg g^{-1})	Total (μg g^{-1})	Soluble (μg g^{-1})	Dialyzable (μg g^{-1})
BPP	79.65	2.28±0.13	2.03±0.18	1.37±0.73	1.74±0.21	1.16±0.28	0.22±0.05	0.73±0.13	0.45±0.29	0.10±0.03	0.81±0.04	0.53±0.08	0.22±0.14
VCP	84.91	4.72±0.13	1.36±0.00	0.40±0.44	2.81±0.06	0.32±0.20	0.11±0.13	1.59±0.08	0.20±0.13	0.17±0.01	1.62±0.12	0.31±0.09	0.10±0.05
VV	86.78	3.31±0.50	1.15±0.36	0.17±0.02	4.14±1.02	0.64±0.43	0.04±0.01	1.18±0.07	0.17±0.04	0.04±0.01	1.45±0.25	0.16±0.10	0.08±0.01
BAO	80.08	1.26±0.26	0.74±0.12	0.64±0.75	2.89±0.22	1.16±0.17	0.21±0.26	0.44±0.08	0.23±0.07	0.21±0.07	0.63±0.03	0.47±0.04	0.26±0.14
MFR	82.00	2.89±0.58	0.74±0.18	0.35±0.31	0.86±0.06	0.47±0.04	0.12±0.18	0.54±0.13	0.15±0.03	0.17±0.05	0.59±0.04	0.25±0.05	0.16±0.05
ZC	89.30	2.46±0.28	0.80±0.03	0.83±0.31	2.18±0.27	0.77±0.05	0.30±0.04	0.58±0.06	0.17±0.09	0.14±0.03	1.36±0.28	0.50±0.09	0.48±0.40
SCF	87.51	2.99±0.09	0.94±0.61	0.53±0.27	1.64±0.01	0.30±0.07	0.24±0.07	0.62±0.06	0.13±0.02	0.05±0.02	1.40±0.14	0.35±0.01	0.22±0.06
VVC	90.11	1.59±0.39	1.48±0.34	0.65±0.25	1.40±0.07	0.96±0.03	0.26±0.03	0.60±0.04	0.28±0.02	0.03±0.00	0.42±0.02	0.44±0.17	0.12±0.06
VC	87.56	2.36±0.30	1.85±0.18	1.06±0.17	3.41±0.08	1.42±0.15	0.38±0.06	0.33±0.02	0.24±0.01	0.14±0.01	0.74±0.02	0.39±0.12	0.17±0.07
PVC	84.84	3.98±0.26	1.41±0.13	0.79±0.20	4.15±0.47	1.14±0.16	0.63±0.08	0.87±0.16	0.32±0.03	0.15±0.05	1.46±0.14	0.67±0.03	0.25±0.11

Iron

Fe content of weaning food ranged from 4.72 µg g^{-1} in the VCP jars to 1.26 µg g^{-1} in the BAO jars (Table 4). As expected, the highest contents of this element were presented in the samples which incorporated both meat and legumes (chickpeas), with statistically significant differences ($p < 0.05$) between this group and the group of weaning foods made exclusively from vegetables, leafy greens or fruits. These results are expected considering that both meat and legumes have high contents of Fe [27, 28]. However, these contents of Fe present in meat-based weaning foods are much lower than those found by us in a previous study in jars which use similar ingredient formulations and are not classified with the attribute of "organic" [19] (Table 5). In that study, where we studied the content of trace elements in weaning foods but not their bioaccessibility, formulations with meat ingredients (chicken, veal, beef and ham) corresponding to three marketed and commercial distributed brands showed average Fe concentrations of 4.74 ± 0.95 (brand A), 6.19 ± 1.46 (brand B) and 6.17 ± 0.54 µg g−1 (brand C), respectively, much greater than those found in the present study. The low percentages of these ingredients in the overall formulation of the jar, below 12 %, may partly justify these results (Table 1).

It can be observed a positive correlation ($r = 0.830$) between protein content and Fe solubility, which means that Fe bioaccessibility increases as the protein content of the sample increases (Fig. 1a). Only BPP would be excluded from this correlation. In this latter, the high solubility of Fe, despite the low protein content, could be due to the presence of vitamin C as ingredient (Table 1). A study made in healthy women [29] has shown an increased dose–response in Fe absorption when small amounts of pork meat (≥50 g) were incorporated into a diet which was high in phytic acid and low ascorbic acid content. This effect, known as "meat factor", suggests that the animal proteins are hydrolyzed to amino acids and small peptides in the process of gastrointestinal digestion, which join to ionic Fe in the intestinal lumen, forming soluble compounds. For some authors, the presence of peptides containing amino acids rich in sulphydryl groups, such as cysteine and histidine, can play an important role in this effect [30–34]. Likewise, Storcksdieck et al. [35] suggest that the “meat factor” may also be due to the presence of peptides with a molecular mass of about 2 kDa, rich in aspartic and glutamic acid, and possibly derived from the acid digestion of myosin. Other in vitro studies carried out with cell lines Caco-2 point out the presence of glycosaminoglycans [36] or L-α-glycerophosphocholine [37], in order to isolate and characterize the substance known as “meat factor”.

In our study, the effect of proteins in the enhanced of Fe bioaccessibility is not only attributable to animal protein. In jars studied in which there was no meat ingredient present, the concentration of soluble Fe also increased with the protein content. Although some authors have suggested that proteins derived from milk, egg and vegetable foods generally decrease the bioavailability of Fe since peptides form insoluble complexes which prevent its uptake by mucosal receptors [38], recent studies have pointed to the contrary effect. Thus, Joshi et al. [39] found that Fe dialysability increased significantly as the protein content increased from 100 to 200 g kg^{-1} in natural corn flour matrices with groundnut protein concentrates. Similarly, Lombardi-Boccia et al. [40] studied the effect of some protein components of beans on Fe dialysability, finding that the highest percentages of Fe dialyzable corresponded to globulin fraction, which also had the highest content of sulfur amino acids such as cysteine. The findings of our study on the promoter effect of the amount of protein in the bioaccessibility of Fe are consistent with these latest researches.

Table 5 Range of concentrations found in a previous study with weaning foods which are not commercialized as "organic" (three brands) [19]

Element	Brand A (µg g−1)	Brand B (µg g−1)	Brand C (µg g−1)
Fe	7.05–1.11	9.20–1.83	6.98–1.01
Zn	1.12–0.30	1.37–0.37	1.12–0.32
Mn	10.40–1.58	16.31–1.18	8.21–1.15
Cu	7.49–0.40	0.89–0.34	0. 65–0.18
Ca	557–54	1228–38	741–163
Mg	201–100	159–81	151–89

Zinc

The highest concentrations of Zn are found in weaning food with meat ingredients as PVC (4.15 µg g^{-1}) and VV (4.14 µg g^{-1}), while the sample composed only of fruit (MFR) had the lowest concentration of this element (0.86 µg g^{-1}; Table 4). In fact, it can be seen a statistically significant correlation (p < 0.01; r = 0.789) between protein content (g 100 g^{-1}) and zinc content (µg g^{-1}) which is logical if one takes into account that the main sources of this element in the diet are food with a high protein content such as meat, cereals, legumes and molluscs [41]. The influence of proteins is also observed in the bioaccessibility of Zn (Zn dialyzable; Fig. 1b). However, this case was only statistically significant (p < 0.01) for concentrations of proteins greater than 2 g 100 g^{-1}; that is to say, only the weaning food that incorporated meat or legumes (chickpeas) in their list of ingredients (Table 4). The presence of some types of proteins in the increased absorption of Zn is well documented [41–44]. do Nascimento da Silva et al. [45] have showed this effect of meat proteins on Zn bioaccessibility to meat with vegetables jars but not to chicken breast with pasta and vegetables jars. In a previous study in school meals, Cámara et al. [25] have reported that the highest percentages of Zn dialyzable correspond to legume and meat dishes. The justification of this effect seems to be very similar to that of Fe; peptides generated during the digestion of proteins chelate Zn ions, forming soluble compounds with this element until it is absorbed by the enterocytes. It appears that the presence of certain amino acids such as cysteine, glycine, histidine and lysine can increase the percentage of Zn dialyzable in a dose-dependent manner. However, this effect also depends on the food matrix and whether or not it is fortified [39].

The similarities between dietary factors that affect the bioaccessibility of Zn and Fe may justify the statistically significant correlation found between soluble Zn–dialyzable Fe ($p < 0.05$; $r = 0.729$) and between dialyzable Zn–dialyzable Fe ($p < 0.05$; $r = 0.745$). That is, many of the dietary factors that promote Fe bioavailability do so with Zn. However, this should not lead us to think that between the two elements there is a synergistic effect upon absorption. It has been widely reported that many trace elements influence each other's absorption by competing in the intestinal lumen for a number of common transporters, among which could be Zip-14 which compete Fe and Zn [46]. Nevertheless, this interaction also depends on the concentrations of both elements initially present in the food. Thus, Agte et al. [47] have found that Zn and Fe did not significantly affect absorption in vegetarian meals of ileostomized humans. Similarly, the low Fe content present in weaning food analyzed in our study (<5 μg g^{-1}) could be why this negative interaction between Zn and Fe was not displayed.

Manganese

Mn content ranged from 1.59 to 0.33 μg g^{-1} (Table 4) and was significantly lower than those found in the previous study discussed above of commercial weaning food without the "organic" label (mean values: brand A = 5.36 ± 2.95 μg g^{-1}; brand B = 6.52 ± 3.71 μg g^{-1}; brand C = 5.15 ± 2.62 μg g^{-1}; Table 5) [19]. The main sources of Mn in the diet are plant foods such as legumes, cereals and dry fruits [48–50] which might justify the higher content of this element in the VCP samples (1.59 μg g^{-1}).

Despite the variability of the data found in the jars tested, the average percentage of bioaccessible Mn was 37.4 % for the solubility assay and 20.2 % for the dialysability assay. Velasco-Reynold et al. [51] have also reported very similar values corresponding to a mean percentage of Mn dialyzed of hospital meals of 22 %. Similarly, do Nascimento da Silva et al.

[45] have found Mn bioaccessible fraction greater than 50 % for jars which contain vegetables such as potatoes, rice flour or wheat flour. For radish roots, lettuce and spinach leaves, it have been indicated percentages of soluble Mn of 20.6, 10.4 and 8.7 %, respectively [52]. Mn bioaccessible values found in our study are thus in agreement with those found in the literature and can be considered moderately high.

A negative and statistically significant correlation (p < 0.01; r = −0.781) is found between Mn dialyzable (µg g^{-1}) and fat content (g 100 g^{-1}) which means that the amount of Mn dialyzable is higher when a lower amount of fat is present in the jar formulation (Fig. 1c). The negative influence of fat on mineral bioavailability has also been reported for other trace elements such as selenium [26]. Probably, a high fat content in the diet can interfere with the enzymes used in this in vitro digestion simulated, which avoid forming peptides of low molecular weight with the Mn present, making it easy to be dialyzed.

Other dietary factors also influencing the bioaccessibility of Mn are Fe and Zn, finding a positive correlation between soluble Mn (µg g^{-1})–soluble Fe (µg g^{-1}) (p < 0.01; r = 0.768) and between soluble Mn (µg g^{-1})– soluble Zn (µg g^{-1}; p < 0.05; r = 0.660). However, the results should not be interpreted in the sense that Fe or Zn promotes the absorption of Mn in the intestinal lumen, since a negative influence of a high Fe content in the diet on the bioavailability of Mn has been widely described [53, 54].

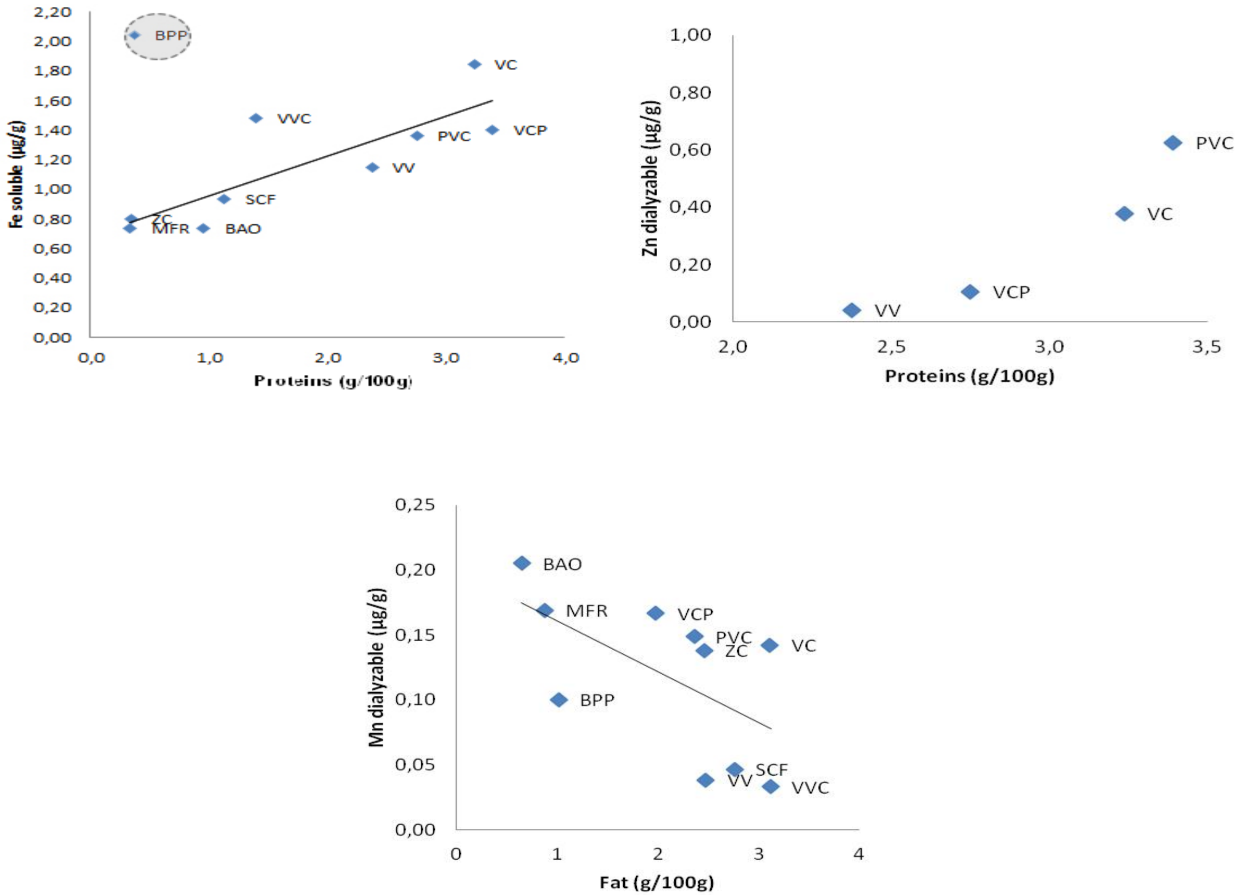


Fig. 1 Correlations between Fe soluble (µg g^{-1})—protein content ▸ (g 100 g^{-1}), **a**); Zn dialyzable (µg g^{-1})—protein content (g 100 g^{-1}), **b**); Mn dialyzable (µg g^{-1})—fat content (g 100 g^{-1}), **c**) for weaning foods studied. *BPP* banana, pear and pomegranate; *VCP* vegetables, chickpeas and pear; *VV* vegetables, veal; *BAO* banana, apple, orange; *MFR* multifruit; *ZC* zucchini creme; *SCF* squash, chamomile flower; *VVC* varied vegetables creme; *VC* vegetables, chicken; *PVC* vegetables, chicken, pasta

Copper

There was a statistically significant correlation between the Cu content (μg g^{-1}) and the Fe content (μg g^{-1}) of the jars studied ($p < 0.01$; $r = 0.801$). It can be also observed in our samples that there was a positive correlation between soluble Cu (μg g^{-1})–dialyzable Fe (μg g^{-1}; $p < 0.01$; $r = 0.818$) and soluble Cu (μg g^{-1})–dialyzable Zn (μg g^{-1}; $p < 0.05$; $r = 0.709$). The existence of a similar positive interaction between Cu and Fe has previously been observed in school meals using an in vitro model—Caco 2 cell line. In this study, a positive correlation was established between Cu retention by cells and Fe content in these school meals [55]. Similarly, Velasco-Reynold et al. [56] have reported a positive correlation between dialysability of Cu and Fe content for duplicate hospital meals.

This in vitro positive interaction among both elements could further justify in vivo results [57]. In rats, it has been observed increases in hemoglobin, hematocrit and serum Fe levels, when for one same amount of Fe (24 μg g^{-1}), the Cu content in diets increased from 0 to 6 μg g^{-1} [58]. Another argument that could justify this positive correlation between bioaccessibility of Cu and bioaccessibility of Fe is like that which occurred between Fe and Zn; the same factors that promote bioavailability of Fe (protein, vitamin C, etc.) do so with Cu. Thus, it has been observed as the content of vitamin C promotes Cu dialyzable amount [56, 59]. It has also been found significant relationships among contents of protein and dialyzable Cu [56] highlighting the importance of peptides derived from the digestion of proteins and some amino acids as ligands capable of forming soluble complexes with Cu in the gastrointestinal tract [60, 61].

Calcium

Ca content in studied jars ranged between 178 and 63 μg g^{-1} (Table 6). These values are lower than those found in a previous study with three brands of conventional weaning foods which ranged between (brand A = 557–54 μg g^{-1}; brand B = 1228–38 μg g^{-1}; brand C = 741–163 μg g^{-1}; Table 5) [19]. Similarly, Zand et al. [20] have also reported a highest mean Ca contents in vegetable (564 μg g^{-1}) and meat (174 μg g^{-1})-based jars. So that, in relation to this element, the organic attribute does not improve the nutritional value of jars.

Ca solubility percentages ranged between 42 % of BAO and 14 % of VCP. It seems that proteins have a depressed effect on Ca bioaccessibility since VCP beside VV jars, with the highest protein contents, also presented the lowest dialysability percentages (7 %). Agree with this, it has been reported a negative influence of protein content in school menus upon Ca retention by Caco-2 cells [55]. Similarly, Anderson [62] reported that a high presence in diet of food rich in protein may promote Ca lost that can exacerbate the risks joined to a diet low in this element.

On the other hand, the Ca dialyzable content (μg g^{-1}) was significantly affected by the Zn dialyzable content (μg g^{-1}; $p < 0.05$; $r = 0.721$); Cu dialyzable content (μg g^{-1}; $p < 0.05$; $r = 736$); and Mg dialyzable content (μg g^{-1}; $p < 0.05$; r = 0.648). This latter positive Ca–Mg interaction has already showed in hospital duplicate meals [63].

Magnesium

The main sources of Mg are plant foods such as legumes, cereals, fruits and vegetables. In the samples studied, Mg contents were around 150 μg g^{-1} (Table 6). This content was very similar to that reported in a previous study by our research group [19], and other studies made in vegetable and meat-based jars in UK [20] and Spain [21]. However, weaning foods of above mentioned studies not classified with the organic attribute so this characteristic does not improve the content of this element in jars.

Mg soluble fraction ranged between 66.47 μg g^{-1} of PVC and 17.63 μg g^{-1} of VVC with the highest values corresponding to jars with meat proteins or legumes such as PVC, VV and VCP. Mean soluble fraction percentage was 25.6 %, slightly lower than the values reported by Anderson et al. [62] who showed that the efficiency of Mg absorption varied from 35 to 45 %.

Similarly, Mg dialyzable percentages were around 18 % for most studied jars which implied a moderate Mg bioaccessibility.

On the other hand, it has previously been found that Mg dialyzable increases significantly with ascorbic acid content [63]. However, in our study, this effect was observed by BAO (25.7 µg g^{-1}) and BPP (22.5 µg g^{-1}) jars, with vitamin C as ingredient (see Table 6), but not for MFR jars (7.7 µg g^{-1}) which showed the lowest levels of this element.

Table 6 Content of de proteins, fat, Ca y Mg total, soluble and dialyzable in the weaning food studied. (mean ± standard deviation)

Sample	Humidity (%)	Ca			Mg			Protein (g $100g^{-1}$)	Fat (g $100g^{-1}$)
		Total (µg g^{-1})	Soluble (µg g^{-1})	Dialyzable (µg g^{-1})	Total (µg g^{-1})	Soluble (µg g^{-1})	Dialyzable (µg g^{-1})		
BPP	79.65	81±17	33±1	15± 1	152±2	32±2	23±1	0.35±0.07	1.02±0.10
VCP	84.91	178±10	22±3	12±5	174±6	41±4	11±7	2.75±0.22	1.98±0.19
VV	86.78	82±10	23±6	5±2	128±5	51±4	2±0	2.37±0.17	2.47±0.15
BAO	80.08	93±6	39±5	12±9	151±5	32±5	26±5	0.95±0.06	0.65±0.19
MFR	82.00	63±3	25±7	14±4	122±3	25±5	8±10	0.34±0.05	0.87±0.13
ZC	89.30	122±9	28±2	22±7	140±12	27±2	25±6	0.34±0.01	2.46±0.13
SCF	87.51	162±15	38±3	25±7	150±17	44±3	23±4	1.13±0.53	2.76±0.33
VVC	90.11	102±6	30±3	12±1	85±2	18±1	15±1	1.40±0.04	3.12±0.80
VC	87.56	66±1	19±2	16±2	131±7	23±2	24±2	3.24±0.30	3.11±0.25
PVC	84.84	83±11	26±1	16±0	153±6	66±1	28±1	3.39±0.24	2.36±0.14

Probabilistic assessment

A probabilistic model approach was developed to estimate the intake level of the elements studied, which derived from consumption of 150–200 g of these organic weaning food. Dietary references intakes (DRI) for Spanish population of 1–3 years old were considered for Fe, Zn, Ca and Mg [64]. In the case of Mn and Cu, because of the lack of data for Spanish population, DRI of USDA were used [65]. The models were developed from values of total and bioaccessible (soluble) trace element content. Also, 95th and 50th percentiles from the generated random values were reported representing the maximum intake level for 95 and 50 % population studied.

As it can be seen in Fig. 2a, the contribution of Fe and Zn to the DRI for jars studied was below 2.2 % considering solubility values and of 4.8 % considering total content values for 50 % population. In the best-case scenario (95th percentile), these contributions did not exceed 8 % of DRI for Fe and Zn, respectively. Similar results were obtained for Ca (Fig. 2b) where contributions to DRI of this mineral did not exceed 3.6 and 1 % of total and bioaccessible contents, respectively, for 50 % population. Only in the case of Mg, results were slightly improved with contributions per jar above 30 % considering total content values and 8 % considering bioaccessible values for 50 % population.

In relation to Mn, though data are inconclusive and just an adequate intake was established for 1–3 year population group, similar trend was observed to what was above described (Fig. 2c). DRI was only correctly accomplished for Cu, considering nearly 100 % of total mineral content data for the 95th percentile and 53 % for the 50th percentile. Likewise, according to bioaccessible data, the percentage of accomplishment of DRI was moderate being 33 % for the 95th percentile.

These low contributions to DRI of Fe, Zn and Ca from consumption of weaning foods have been previously reported [19]. The existence of low contributions of these elements in the studied weaning foods entails a risk in population groups which, precisely, need high Fe, Zn and Ca intakes during this stage for an adequate development and growing.

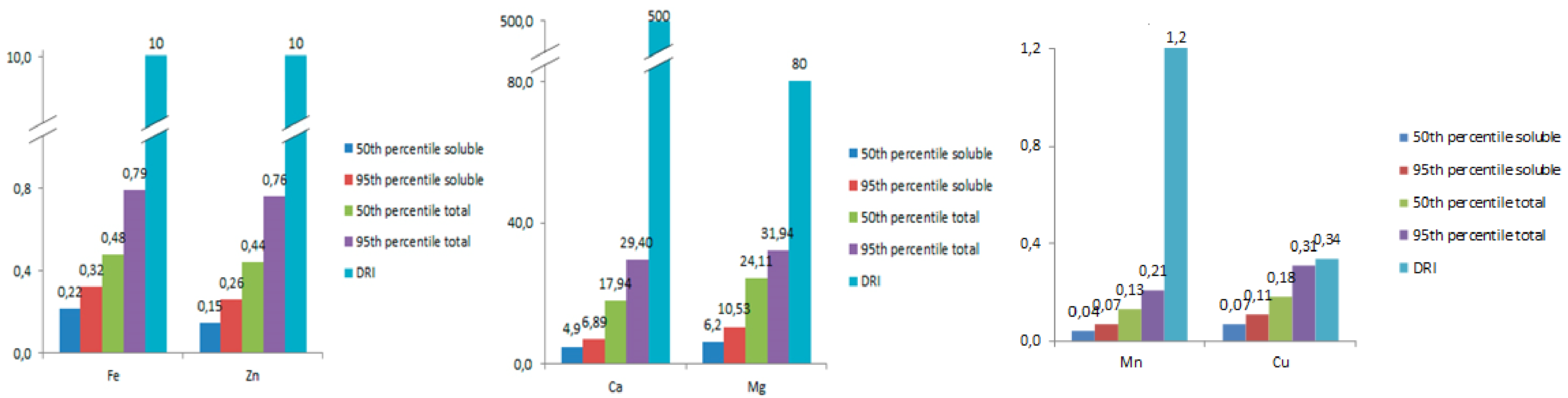


Fig. 2 Estimation of the intake level of Fe, Zn (**a**), Ca, Mg (**b**), Mn and Cu (**c**) derived from consumption of 150–200 g of organic weaning food. Contributions to dietary references intakes (dates are expressed in mg)

Conclusions

The concentrations of trace elements analyzed in the jars studied, marketed under the ecological attribute, are found in many cases below those found in similar samples that are not sold with this feature. Furthermore, in the present study, it has been observed as the presence of certain types of protein, both animal and vegetable, can improve the bioaccessibility of Fe, and to a lesser extent, the bioaccessibility of Zn. Vitamin C may also promote absorption of Fe in the case where the protein concentration is low. Conversely, fat shows a depressing effect on the bioaccessibility of Mn. Some positive interactions between elements previously documented in the literature such as that between Fe and Cu was also observed in this study. The development of a probabilistic study to determine the degree of contribution to the DRI for Fe, Zn, Ca, Mg, Mn and Cu from the consumption of these jars shows a low percentage, especially in the case of Fe, Zn and Ca. According to the analyzed samples, the consumption of these organic foods does not guarantee better nutritional value.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or living animal subjects.

References

1. Paulose B, Datta SP, Rattan RK, Chhonkar PK (2007) Effect of amendments on the extractability, retention and plant uptake of metals on a sewage irrigated soil. Environ Pollut 146:19–24

2. Juraske R, Mutel CL, Stoessel F, Hellweg S (2009) Life cycle human toxicity assessment of pesticides: comparing fruit and vegetable diets in Switzerland and the United states. Chemosphere 77(7):939–945

3. Quijano L, Yusà V, Font G, Pardo O (2016) Chronic cumulative risk assessment of the exposure to organophosphorus, carbamate and pyrethroid and pyrethrin pesticides through fruit and vegetables consumption in the region of Valencia (Spain). Food Chem Toxicol 89:39–46

4. Lemos J, Sampedro MC, de Ariño A, Ortiz A, Barrio RJ (2016) Risk assessment of exposure to pesticides through dietary intake of vegetables typical of the Mediterranean diet in the Basque Country. J Food Compos Anal 49:35–41

5. Radwan MA, Salama AK (2006) Market basket survey for some heavy metals in Egyptian fruits and vegetables. Food Chem Toxicol 44:1273–1278

6. Waegeneers N, Pizzolon JC, Hoenig M, de Temmerman L (2009) The European maximum level for cadmium in bovine kidneys is in Belgium only realistic for cattle up to two years of age. Food Addit Contam A 26(9):1239–1248

7. De Souza Araújo D, Bastos da Silva AM, De Andrade Lima L, Vasconcelos MA, Andrade SA, Sarubbo LA (2014) The concentration of minerals and physicochemical contaminants in conventional and organic vegetables. Food Control 44:242–248

8. Koh E, Wimalasiri K, Renaud E, Mitchell AE (2008) A comparison of flavonoids, carotenoids and vitamin C in commercial organic and conventional marinara pasta sauce. J Sci Food Agric 88:344–354

9. Lester GE, Manthey JA, Buslig BS (2007) Organic vs conventionally grown Rio Red whole grapefruit and juice: comparison of production inputs, market quality, consumer acceptance, and human health-bioactive compounds. J Agric Food Chem 55:4474–4480

10. Bosscher D, Deelstra H, Van Caillie-Bertrand M (2002) Recent advances in the development of infant formulas: mimicking the effects of breast feeding. Nutrition 18(6):522–523

11. Moreno-Rojas R, Cañal-Ruíz C, Amaro-López MA, Cámara-Martos F (2015) Probabilistic assessment of the intake of mineral and trace elements by consumption of infant formulas and processed cereal-based food in Spain. CYTA-J Food 13(2):243–252

12. Lesniewicz A, Wroz A, Wojcik A, Zyrnicki W (2010) Mineral and nutritional analysis of Polish infant formulas. J Food Compost Anal 23:424–431

13. Molska A, Gutowska I, Baranowska-Bosiacka I, Nocen I, Chlubek D (2014) The content of elements in infant formulas and drinks against mineral requirements of children. Biol Trace Elem Res 158:422–427

14. Vieira da Silva S, Mattana P, Bizzi CA, dos Santos Pereira, Richards NS, Smanioto Barin J (2013) Evaluation of the mineral content of infant formulas consumed in Brazil. J Dairy Sci 96(6):3498–3505

15. Sola-Larrañaga C, Navarro-Blasco I (2006) Preliminary chemometric study of minerals and trace elements in Spanish infant formulae. Anal Chim Acta 555:354–363

16. Bermejo P, Peña E, Domínguez R, Bermejo A, Fraga JM, Cocho JA (2000) Speciation of iron in breast milk and infant formulas whey by size exclusion chromatography-high performance

liquid chromatography and electrothermal atomic absorption spectrometry. Talanta 50(6):1211–1222

17. Ruíz MC, Alegría A, Barberá R, Farré R, Lagarda MJ (1996) Calcium, magnesium, sodium, potassium and iron content of infant formulas and estimated daily intakes. J Trace Elem Med Biol 10:25–30

18. Navarro-Blasco I, Álvarez-Galindo JI (2004) Selenium content of Spanish infant formulae and human milk: influence of protein matrix, interactions with other trace elements and estimation of dietary intake by infants. J Trace Elem Med Biol 17:277–289

19. Moreno-Rojas R, Cañal-Ruíz C, Benajiba N, Cámara-Martos F (2013) Probabilistic assessment of the intake of trace elements by consumption of weaning foods in Spain. Ecol Food Nutr 52(3):251–265

20. Zand N, Chowdhry BZ, Zotor FB, Wray DS, Amuna P, Pullen FS (2011) Essential and trace elements content of commercial infant foods in the UK. Food Chem 128:123–128

21. Mir-Marqués A, González-Masó A, Cervera ML, de la Guardia M (2015) Mineral profile of Spanish commercial baby food. Food Chem 172:238–244

22. ESPGHAN Committee on Nutrition (2008) Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 46:99–110

23. Cámara-Martos F, Pérez-Rodríguez F, Amaro-López MA, Moreno-Rojas R (2011) In: Betancourt AI, Gaitán HF (eds) Micronutrients: sources, properties and health effects, 1st edn. Nova Science Publishers, New York

24. Cámara-Martos F, Marval-León J, Moreno-Rojas R (2015) In: Morrison W (ed) Selenium: dietary sources, properties and role in human health, 1st edn. Nova Science Publishers, New York

25. Cámara F, Amaro MA, Barberá R (2005) Bioaccessibility of minerals in school meals: comparison between dialysis and solubility methods. Food Chem 92(3):481–489

26. Marval-León JR, Cámara-Martos F, Amaro-López MA, Moreno-Rojas R (2014) Bioaccessibility and content of Se in fish and shellfish widely consumed in Mediterranean countries: influence of proteins, fat and heavy metals. Int J Food Sci Nutr 65(6):678–685

27. De Castro Cardoso Pereira PM, dos Reis Baltazar Vicente AF (2013) Meat nutritional composition and nutritive role in the human diet. Meat Sci 93:586–592

28. Canniatti Brazaca SG, Da Silva FC (2003) Enhancers and inhibitors of iron availability in legumes. Plant Foods Hum Nutr 58:1–8

29. Baech SB, Hansen M, Bukhave K et al (2003) Nonheme-iron absorption from a phytate-rich meal is increased by the addition of small amounts of pork meat. Am J Clin Nutr 77:173–179

30. Amaro-López MA, Cámara-Martos F (2004) Iron availability: an update review. Int J Food Sci Nutr 55:597–606

31. Mulvihill B, Morrisey P (1998) Influence of the sulphydryl content of animal proteins on in vitro bioavailability of non-heme iron. Food Chem 61:1–7

32. Kirwan FM, O'Connor I, Morrisey PA, Flynn A (1993) Effect of myofibrillar muscle proteins on in vitro bioavailability of iron. Proc Nutr Soc 52:21a

33. Taylor PG, Martinez-Torres C, Romano EL, Layrisse M (1986) The effect of cysteine-containing peptides released during meat digestion on iron absorption in humans. Am J Clin Nutr 43:68–71

34. Layrisse M, Martinez-Torres C, Leets I, Taylor P, Ramirez J (1984) Effect of histidine, cysteine, glutathione or beef on iron absorption in humans. J Nutr 114:217–223

35. Storcksdieck S, Bonsmann G, Hurrell RF (2007) Iron—binding properties, amino acid composition, and structure of muscle tissue peptides from in vitro digestion of different meat sources. J Food Sci 72(1):S19–S29

36. Huh EC, Hotchkiss A, Brouillette J, Glahn RP (2004) Carbohydrate fractions from cooked fish promote iron uptake by Caco-2 cells. J Nutr 134:1681–1689

37. Armah CN, Sharp P, Mellon FA (2008) L-alpha-glycerophosphocholine contributes to meat's enhancement of nonheme iron absorption. J Nutr 138:873–877

38. Berner LA, Miller DD (1985) Effects of dietary proteins on iron bioavailability—a review. Food Chem 18:47–69

39. Joshi V, Thatte P, Prakash J, Jyothi A (2014) Effect of oilseed protein concentrates and exogenous amino acids on the dialysability of iron and zinc. Food Sci Technol-LEB 59:540–546

40. Lombardi-Boccia G, Carbonaro M, Di Lullo G, Carnovale E (1994) Influence of protein components (G1, G2 and albumin) on Fe and Zn dialysability from bean. Int J Food Sci Nutr 45:183–190

41. Cámara-Martos F, Moreno-Rojas R (2016) Zinc: properties and determination. In: Caballero B, Finglas P, Toldrá F (eds) The encyclopedia of food and health 5:638–644

42. Bel-Berrat S, Stammers AL, Warthon-Medina M, Hall Moran V, Iglesia-Altaba I, Hermoso M, Moreno LA, Lowe NM (2014) Factors that affect zinc bioavailability and losses in adult and elderly populations. Nutr Rev 72(5):334–352

43. Cámara F, Amaro MA (2003) Nutritional aspect of zinc availability. Int J Food Sci Nutr 54(143–151):43

44. Velasco-Reynold C, Navarro-Alarcón M, López-G de la Serrana H, Pérez-Valero V, López-Martínez MC (2008) In vitro determination of zinc dialyzability from duplicate hospital meals: influence of other nutrients. Nutrition 24:84–93

45. do Nascimento da Silva E, Perriello Leme AB, Cidade M, Cadore S (2013) Evaluation of the bioaccessible fractions of Fe, Zn, Cu and Mn in baby foods. Talanta 117:184–188

46. Iyengar V, Pullakhandam R, Madhavan Nair K (2012) Coordinate expression and localization of iron and zinc transporters explain iron–zinc interactions during uptake in Caco-2 cells: implications for iron uptake at the enterocyte. J Nutr Biochem 23:1146–1154

47. Agte V, Jahagirdar M, Chiplonkar S (2005) Apparent absorption of eight micronutrients and phytic acid from vegetarian meals in ileostomized human volunteers. Nutrition 21:678–685

48. Leblanc JC, Guérin T, Noël L, Calamassi-Tran G, Volatier JL, Veger P (2005) Dietary exposure estimates of 18 elements from the 1st French total diet study. Food Addit Contam 22:624–641

49. Santos EE, Lauria DC, Porto da Silveira CL (2004) Assessment of daily intake of trace elements due to consumption of foodstuffs by adult inhabitants of Rio de Janeiro city. Sci Total Environ 327:69–79

50. Noël L, Leblanc JC, Guérin T (2003) Determination of several elements in duplicate meals from catering establishments using closed vessel microwave digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake. Food Addit Contam 20:44–56

51. Velasco-Reynold C, Navarro-Alarcón M, López-G de la Serrana H, Pérez-Valero V, López-Martínez MC (2008) Total and dialyzable levels of manganese from duplicate meals and influence of other nutrients: estimation of daily dietary intake. Food Chem 109:113–121

52. Intawongse M, Dean JR (2006) Uptake of heavy metals by vegetable plants grown on contaminated soil and their bioavailability in the human gastrointestinal tract. Food Addit Contam 23:36–48 Thompson K, Molina R, Donaghey T, Brain JD, Wessling-Resnick M (2006) The influence of high iron diet on rat lung manganese absorption. Toxicol Appl Pharm 210:17–23

54. Aschner M, Erikson KM, Dorman DC (2005) Manganese dosimetry: species differences and implications for neurotoxicity. Crit Rev Toxicol 35:1–32

55. Cámara F, Barberá R, Amaro MA (2007) Calcium, iron, zinc and copper transport and uptake by Caco-2 cells in school meals: influence of protein and mineral interactions. Food Chem 100(3):1085–1092

56. Velasco-Reynold C, Navarro-Alarcón M, López-G de la Serrana H, Pérez-Valero V, López-Martínez MC (2008) Analysis of total and dialyzable copper levels in duplicate meals by ETAAS: daily intake. Eur Food Res Technol 227:367–373

57. Sharp P (2004) The molecular basis of copper and iron interactions. Proc Nutr Soc 63:563–569

58. Ramírez-Cárdenas L, Brunoro Costa NM, Pinheiro Reis F (2005) Copper–iron metabolism interaction in rats. Nutr Res 25(1):79–92

59. Navarro M, Wood RJ (2003) Plasma changes in micronutrients following a multivitamin and mineral supplement in healthy adults. J Am Coll Nutr 22(2):124–132

60. Bosscher D, Van Cauwenbergh R, Robberecht H, Van Caillie-Bertrand M, Deelstra H (2002) Daily dietary iron, zinc and copper intake of infants in Belgium. Eur Food Res Technol 215(4):275–278

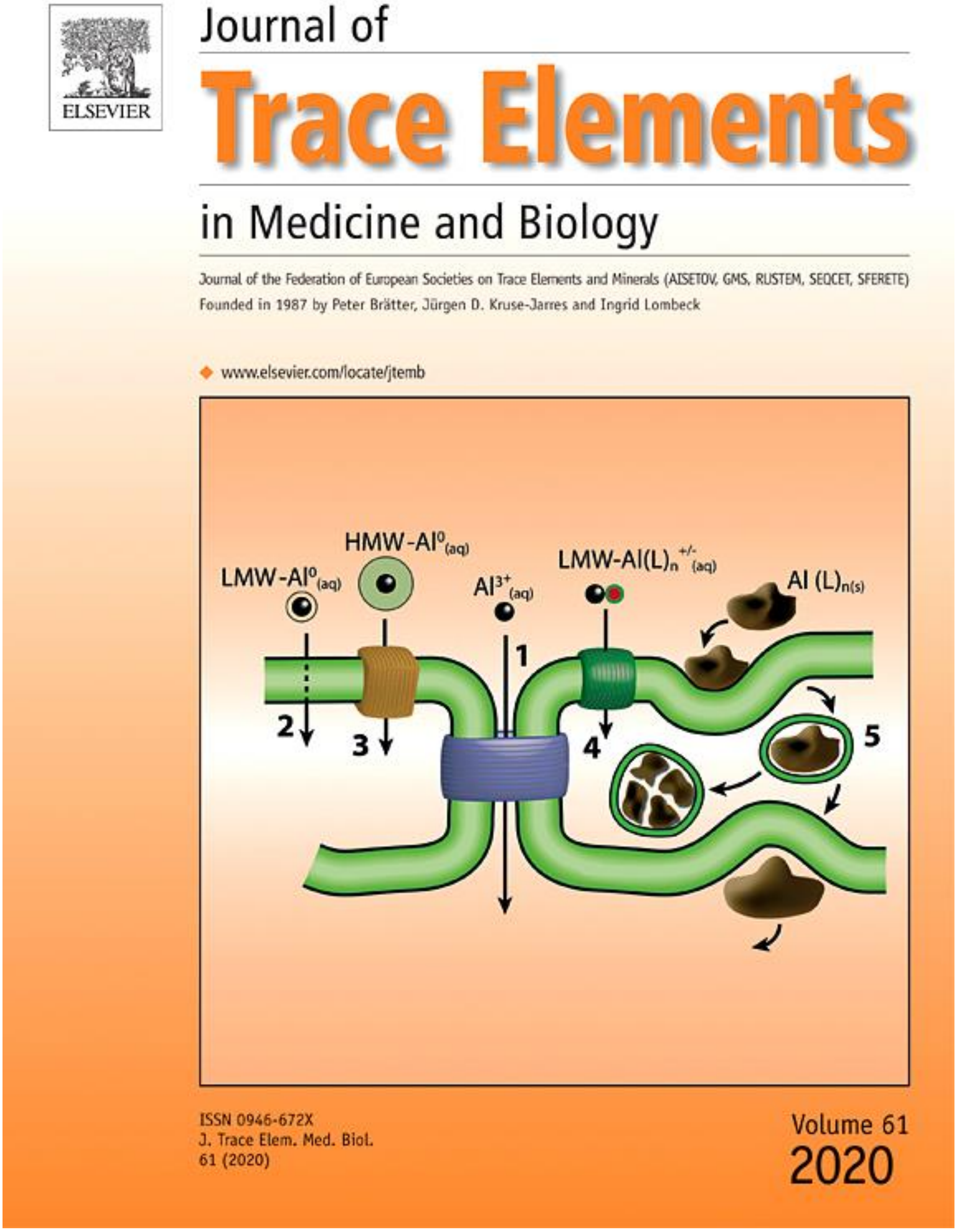
61. Dendougui F, Schwedt G (2002) Use of an ion selective electrode to determine the complexing of copper in food extracts dependent upon the pH. Eur J Food Res Technol 215:76–82

62. Anderson JJB (2004) Minerals. In: Mahan LK, Escott-Stump S (eds) Food, nutrition, and diet therapy, 11th edn. Saunders, Philadelphia, pp 135–143

63. Velasco-Reynold C, Navarro-Alarcón M, López-Gª de la Serrana H, Pérez-Valero V, Agil A, López-Martínez MªC (2010) Dialysability of magnesium and calcium from hospital meals: influence exerted by other elements. Biol Trace Elem Res 133:313–324

64. FESNAD (2010) Ingestas Dietéticas de Referencia (IDR) para la Población Española. EUNSA, Spain

65. United States Department of Agriculture (USDA) (2010) Dietary guidelines for Americans 2010, 7th edn. Government Printing Office, Washington, DC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3.4. CAPÍTULO 4: Selenium and cadmium in bioaccessible fraction of organic weaning food: Risk assessment and influence of dietary components

F. Cámara-Martos, AM. Ramírez-Ojeda, M. Jiménez-Mangas, J. Sevillano-Morales, R. Moreno-Rojas

Departamento de Bromatología y Tecnología de los Alimentos, Universidad de Córdoba, Campus Universitario de Rabanales, Edificio C-1, 14014, Córdoba, Spain

Corresponding author at: Departamento de Bromatología y Tecnología de los Alimentos, Universidad de Córdoba, Campus Universitario de Rabanales, Edificio Charles Darwin C-1, 14014, Córdoba, Spain.

E-mail address: bt2camaf@uco.es (F. Camara-Martos).

ABSTRACT

Background: The tendency of some sectors of the population to consume organic food has also come to include baby food. Nevertheless, it is necessary to develop studies to support the true nutritional and toxicological value of these products, making special emphasis in several trace elements. To our knowledge, no studies have been conducted on this type of organic food.

Methods: Weaning foods with different formulations categorized as organic were analyzed to determine Se and Cd contents as well as its bioaccesibility. The analyses were conducted by electro thermal atomic absorption spectroscopy (ET – AAS) after the treatment of the samples with acid mineralization. Besides, macronutrient analyses (protein, fat and dietary fiber) were also developed. Finally, a novelty statistic approach such as @Risk was used to evaluate contributions to DRI or PTWI of Se and Cd derived for consumption of these weaning foods.

Results: Se content ranged between 2.44–15.4 μg Kg ^{1}. Samples with meat ingredients showed the highest Se contents, while weaning foods consisting of fruits or vegetables presented the lowest concentrations. Se bioccessible concentration ranged between 1.90–4.35 μg Kg^{-1} with a greater uniformity amongst analyzed samples. Regarding Cd, concentrations of this heavy metal ranged between 1.23 and 3.64 μg Kg^{-1}. Furthermore, Cd bioaccessibility of organic weaning foods ranged between 0.17 and 1.38 μg Kg^{-1}. The solubility of all samples studied was around 20% from the initial Cd concentration. A negative statistical correlation between fat content – Cd bioaccesible ($p < 0.05$; $r = -0.756$) and Cd content – Se bioaccesible ($p < 0.05$; $r = -0.777$) were also found.

Conclusions: Cd concentrations are considerably lower than those reported in weaning formulas which were not categorized as organic. On the other hand, the analysed organic jars did not represent a significant source of Se. The probabilistic assessment developed, showed that contributions to DRI of Se for infants 1–3 years old by consumption of these weaning foods, are excessively low (15% at best).

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1. Introduction

Currently, organic food production is one of the fastest growing markets, with an increase of around 250% in the last 10 years [1]. The principal reason is that organic agriculture can enhance human and environmental health because there is no use of synthetic fertilizers or pesticides. Nevertheless, the lack of confidence in the labeling of many organic products, the reduced half-life of these foods compared to conventional ones and higher production cost, make it is necessary to develop studies to support the true nutritional and toxicological value of these products [1]. Research data on the nutritional benefits of organic food compared to their non-organic homologues is scarce and contradictory [2,3].

The tendency of some sectors of the population to consume organic food has also come to include baby food. However, the nutritional composition of this baby food should be monitored very strictly as these infant foods must provide high biological value proteins, energy and micronutrients. One of these micronutrients considered essential to the human body is selenium (Se). This trace element plays an important role in several physiological functions, such as forming part of enzymes like glutathione peroxidase [4], thioredoxin reductase [5] and iodothyronine-5 deiodinase [6]. A low maternal Se status during pregnancy has been related to fetal malformations, like neural tube defects [7], and is disadvantageous for cognitive development in infants and toddlers [8,9]. However, Se may also be toxic for many organisms when it is presented in high concentrations, being one of the chemical elements in which there is less difference between essential and toxic levels [10].

On the other hand, cadmium (Cd) is a ubiquitous heavy metal, widespread in agriculture as a consequence of the use of pesticides. It is expected that organic agricultural practices result in a lower content of Cd in food produces. In an infant´s early life, low-level Cd exposure through breast milk could induce oxidative stress [11]. Besides, Cd could be stored in different organs (lungs, kidney, digestive system, bone tissue, gonads) and remains in a child's body until adulthood [12] (up to 30 years). In addition, it has also been identified as a potent neurotoxin [13]. Finally, data from animal experiments show that the gastrointestinal absorption of Cd in newborns is significantly higher than in adults [14].

In order to evaluate the nutritional and toxicological value of food, it is necessary not only to determine the trace element or heavy metal concentration initially present but also evaluate its bioaccessibility. This term refers to the amount of the inorganic element (trace element or heavy metal) that is found soluble in the intestinal lumen after an *in vitro* simulated digestion; therefore, this element is available to be absorbed by the enterocytes. This bioaccessibility is influenced by the presence of other components present in the food matrix (fiber fractions, peptides from protein digestion, vitamins, etc.). Therefore, it is of foremost relevance when developing food formulations to know how food components influence bioaccessibility by increasing or decreasing trace element absorption.

Several studies have been made concerning Se and Cd bioaccessibility in food matrices such as pulses [15], cereals [16], and fish [17] in the case of Se, and vegetables [18], shellfish [19,20], and pulses [15] with Cd. However, as far as we know, only one study was found which assessed bioaccessibility of Cd in weaning foods, nevertheless its ingredients were not labeled as organic [14]. Given the above, the objective of this article was i) to determine the bioaccessibility and the total content of Se and Cd present in weaning food categorized with the "organic" attribute ii) to study the influence of other food components such as protein, fiber and fat in the bioaccessibility of these elements; iii) to develop a probabilistic/risk assessment study of Se and Cd intake by consumption of these organic weaning foods with a novel approach (@Risk) in order to evaluate the nutritional/toxicological value of these products.

2. Materials and methods

2.1. Materials and reagents

All reagents were of analytical-reagent grade. Ultrapure water (18 MΩ/SCF) prepared with a Milli-Q Reference Water Purification (Millipore, Madrid, Spain) was used throughout experiments. All glassware and plastic containers were soaked in 10% nitric acid over- night and rinsed three times with de-ionized water prior to use. Nitric acid (65%), hydrochloric acid (35%) and sulphuric acid (97%) were obtained from Panreac (Barcelona, Spain). Sodium bicarbonate (97%) and sodium hydroxide were obtained from Scharlab (Barcelona, Spain).

Magnesium nitrate hexahydrate (98%) and magnesium oxide (98%) were obtained from Alfa Aesar (Kandel, Germany).

Digestive enzymes and bile salts were supplied by Sigma-Aldrich Co. (St. Louis, MO). The working solutions of these enzymes were prepared immediately before use. Pepsin solution was obtained by dissolving 3.2 g of pepsin (P-7000 from porcine gastric mucosa) in 20 ml of HCl (0.1 M). The solution of pancreatin and bile salts were prepared by dissolving 0.6 g of pancreatin (P-3292 from porcine pancreas) and 3.9 g of bile salts (B-8756 of porcine origin) in 150 ml of 0.1 M $NaHCO_3$.

Enzymes used for fibre assays (α-amylase heat stable; protease from *Bacillus licheniformis*; amyloglucosidase from Aspergillus *niger*) were obtained from Sigma-Aldrich Co. (St. Louis, MO). Standard solutions for measuring the elements Se and Cd were prepared immediately before use by dilution with distilled deionised water of 1000 mg/L standard solutions (Scharlau Chemie, Barcelona, Spain).

2.2. Samples

Se and Cd contents were analyzed in 10 commercialized weaning foods characterized with the “organic” attribute (jars of 200 g). These weaning foods were received at the University of Cordoba (Spain) proceeding from a factory specialized on the manufacture and marketing of organic food. Upon arrival at the laboratory, samples were poured in plates, freezed – dried and packed in polypropylene vacuum bags, until required for analyses.

All ingredients used in the formulation of different weaning food are described in Table 1. For mineral content determination, a total of 50 samples were analyzed, corresponding to one sample taken from five different jars for each type of weaning food. In order to get a representative sample, this procedure was repeated for the bioaccesibility assay. The total samples analyzed in both determinations were 100.

Table 1
Ingredients used in the formulation of weaning food analyzed (data supplied by manufacturer).

	Code	Ingredients
Banana, Pear and Pomegrate	BPP	Fruit 97.5% (banana, pear, pomegranate juice, lemon juice), flour 2.5%, vitamin C.
Vegetables, Chickpeas and Pear	VCP	Cooking water and vegetables 45.5% (potato, squash, tomato, onion, green beans), chickpeas 6%, pear 3%, flour 2.5%, extra virgin olive oil 2%, paprika0.14%.
Vegetables, Veal	VV	Vegetables 67.5% (tomato, potato, onion, peas, carrot), veal 12%, cooking water, flour 2.5%, extra virgin olive oil and spices 2%.
Banana, Apple, Orange	BAO	Fruit 100% (banana, apple, orange juice, lemon juice), vitamin C.
Multifruit	MFR	Fruit 95.5% (banana, apple, orange juice, for, grape juice, lemon juice), carrot juice 4.5%, vitamin C.
Zucchini Creme	ZC	Vegetables 75% (zucchini, potato, leek, onions), cooking water, extra virgin olive oil.
Squash, Chamomole Flower	SCF	Cooking water and vegetables 69.5% (squash, potato, onion, leek), extra virgin olive oil 2%, peppermint and chamomile flower.
Varied Vegetables Creme	VVC	Cooking water and vegetables 72% (potato, leek, green beans, onion, zucchini) and extra virgin olive oil 2%.
Vegetables, Chicken	VC	Cooking water and vegetables 56% (potato, tomato, onion, red pepper), chicken 12%, extra virgin olive oil 2% parsley and spices.
Vegetables, Chicken, Pasta	PVC	Cooking water and vegetables 51% (potato, tomato, onion), chicken 12%, wholemeal pasta 4%, extra virgin olive oil 2% and spices.

2.3. Procedure for in vitro gastrointestinal digestion

The bioaccessible fraction of weaning foods, was obtained through an *in vitro* process of gastrointestinal digestion simulated based on the one described by Cámara et al. [21]. The procedure consisted on measuring 3 g of lyophilized sample of weaning food and adjusting pH to 2.0 using 6 N HCl. The first stage required a pepsin – HCl digestion in which 0.5 g of pepsin solution for each 100 g of homogenized was added (corresponding to 0.125 g of porcine pepsin by 3 g of lyophilized sample). The mixture was then incubated for 2 h at 37 °C in a shaking water bath (HSB-2000 Shaking Bath; E-Chrom Tech CO., LTD, Taipei, Taiwan). After this time, to stop gastric digestion, the sample was maintained for 10 min in an ice bath.

Following, the pH was adjusted to 5 by adding 1 M $NaHCO_3$ to continue with intestinal digestion step. Then, 6.2 mL of a mixture of pancreatin and bile salts (corresponding to 0.025 g of pancreatin and 0.160 g of bile salts by 3 g of lyophilized sample) was added to each test tube, which was incubated for 2 h more. After Intestinal digestion, flasks with samples were submerged for 10 min in an ice bath to stop the action of this mixture of enzymes.

Finally the pH was adjusted to 7.2 with 0.5 M NaOH. Aliquots of the digested sample were transferred to polypropylene centrifuge tubes (50 ml, Costar Corning Europe, Badhoevedorp, The Netherlands) and these were centrifuged for 1 h at 4000 rpm and 4 °C. (eppendorf Centrifuge 5810 R) Then, the supernatant (soluble fraction) was collected, its organic matter was destroyed, and the mineral content was measured by atomic absorption spectrometry.

2.4. Trace element determination

To determine Se and Cd content of weaning foods 1 g of lyophilized sample was weighed in a porcelain crucible. Samples were treated with 1.5 mL of ashing aid suspension (20% w/v $MgNO_3$ and 2% w/v MgO) and 5 mL of 7 M HNO_3 in order to avoid Se volatilization. The mixture was evaporated on a heating plate at 80 ºC until total dryness. Subsequently, samples were incinerated in a muffle furnace at 460 ºC for 15 h. The ash was bleached after cooling by adding 2.5 mL of 2 N HNO_3, drying on thermostatic hotplates, and maintaining in a muffle furnace at 460 °C for 1 h more. Ash recovery was performed with 1 mL of HCl 6 N, making up to 10 mL with deionised water. Se and Cd content present in bioaccessible fraction obtained in the previous process were determined in a similar way.

A Perkin-Elmer model AAnalyst 600 atomic absorption spectrometer equipped with a Perkin-Elmer model FIAS 400 hydride generation system and an autosampler was used for Se measurements. Analytical determinations of Cd were measured by graphite furnace atomic absorption spectrometry in a Perkin Elmer model AAnalyst 600 equipped with a Zeeman furnace module and an autosampler AS-800, controlled by the WinLab 32 software. Electrodeless discharge lamps, operated from an external power supply were used. The instrumental parameters are derived from previous optimization works [17,22] (Tables 2 and 3). The accuracy and precision of the different analytical techniques used while determining Se and Cd concentrations were validated by recovery experiments using CRMs (Table 4).

AOAC methods (2005) were used to determine the proximate composition: defatting in a Soxhlet apparatus with petroleum ether for crude fat (920.85) and micro-Kjeldahl for protein (920.87). Briefly, to Kjeldahl method 0.5 g of lyophilized sample with a catalyst pellet was placed in digestion flask and 20 mL of concentrated H_2SO_4 were added. The mixture was heated in a digester until solution clears. After cooling with 70 mL of deionised water, the solution was distilled with a small quantity of NaOH, which converts the ammonium salt to ammonia. The ammonia gas is led into a trapping solution of HCl 0.1 N and determined by back titration with NaOH solution 0.1 N. The protein concentration was calculated from the nitrogen values using a conversion factor of 6.25. For the determination of fat content, 3–5 g of lyophilized sample were weighed and wrapped in a paper filter, and subsequently transferred into a Soxhlet liquid/solid extractor with petroleum ether during 1 h. After fat extraction, samples were dried in desiccators and weighed.

Dietary fibre (total, soluble and insoluble) were determinated by the enzymatic gravimetric method of the AOAC (991.43) [23]. The basis of this method is the isolation of dietary fiber from the rest of components by enzymatic digestion (Step 1: amylase – T=95°C – t =30 min; Step 2: protease – T=60°C – t =30 min; Step 3: amyloglucosidase –T=60°C – t =30 min). After all these steps, ethanol is added to precipitate the soluble fibre (this step is avoided in the insoluble fiber determination). Finally, the residue is filtered in crucibles with 0.5 g of celite, washed with 78% and 95% ethanol and acetone and dried to measure the weight of residue.

Table 2

Instrumental conditions for determination of Cd (λ =228.8 nm) by ET – AAS.

Step	Temperature (°C)	Ramp (s)	Hold (s)	Argon flow (mL/min)
1	130	10	45	300
2	300	10	20	300
3	800	10	20	300
4	1450	0	4	0
5	2600	2	2	300

ET – AAS: Electrothermal Atomic Absorption Spectroscopy.

Table 3

Instrumental conditions for determination of Se (λ =196 nm) by HG – ET – AAS.

Step	Temperature (°C)	Ramp (s)	Hold (s)	Argon flow (mL/min)
1	250	1	50	0
2	250	1	20	250
3	1950	0	5	0
4	2300	1	3	250

Step	Time (s)	Speed of pump 1 (rpm)	Speed of pump 2 (rpm)
Prefill	15	100	0
Fill	15	100	0
Fill	5	100	80
Injection	30	0	80

FIAS flow injection program used for the hydride generation-AAS measurements.

Prefill step: FIAS autosampler sampling tube rinsed with simple solution (only done for the first replicate).

HG – ET –AAS: Hydride Generation with Electrothermal Atomic Absorption Spectroscopy.

Table 4

Analysis of certified reference materials for Se and Cd.

CRM	Se (mg/Kg)			Cd (mg/Kg)		
	Found	Certified	Recovery (%)	Found	Certified	Recovery (%)
Spinach leaves (SRM – 1570)	0.111 ± 0.007	0.117 ± 0.009	95			
Bovine liver (BCR – 185R)	1.68 ± 0.14	1.64 ± 0.04	98	0.530 ± 0.003	0.544 ± 0.017	97

2.5. Statistics and risk assessment

The data were analyzed using SPSS 15.0 (IBM, Armonk, NY). In order to validate the normality of the data obtained, the Shapiro-Wilks test was used. Later, Pearson's correlation (parametric conditions) was used for determining the dependence between variables. Significant differences were considered when $p < 0.05$.

A probabilistic model was developed to estimate the intake level for Se and Cd derived from consumption of weaning foods. The model here developed followed a probabilistic approach in which variables were described by probability distributions. They were fitted to concentration data obtained in our study for each element (total element concentration and bioaccessible element concentration). Furthermore, in order to estimate the intake level, serving size was considered. However, since no data were found concerning the consumption patterns of this type of product, we assumed a serving size ranging from 150 to 200 g for infants (one jar) between 1 and 3 years old, which was defined by a uniform distribution in the probabilistic model; meaning that all values in that range had the same probability to occur.

The probability distributions describing the Se and Cd concentration data were fitted by using @Risk v7.5 (Palisade, Newfield, NY, USA). The simulation was run using 100,000 iterations for each element. The goodness of fit to data was assessed by using different statistical tests which corresponded to Akaike Information Criterion (AIC) test and Chisquare test. These statistical tests allow researchers to give a guess of how well the fitted distribution described the observed data. In addition, the visual analysis was equally considered to assess the fit of the probability distributions to intake data.

3. Results and discussion

3.1. Selenium

Se content in organic weaning foods ranged between 2.5–15.4 µg Kg^{-1} (Table 5). In accordance with previous studies that show protein foods are the main dietary sources of Se [24], samples with meat ingredients such as PVC (15.4 µg Kg^{-1}), VC (12.8 µg Kg^{-1}) and VV (8.4 µg Kg^{-1}) showed the highest Se contents, while weaning foods consisting of fruits or vegetables presented the lowest concentrations (SCF, ZC and MFR: 2.5 µg Kg^{-1}). In fact, a positive significant correlation ($p < 0.01$; r = 0.817) was observed between Se content (µg · Kg^{-1}) and protein content (mg 100 g^{-1}). From the data, it is apparent that vegetable based weaning foods generally had a poorer quality in relation to Se than meat based. On the other hand, the results found for all recipes are higher than those reported by Zand et al. [25], who showed values below the limit of quantification (< 2.4 µg Kg^{-1}) for eight brands of infant complementary food from the United Kingdom, in which some of the ingredients used were categorized as organic.

Similar values have been reported by Ruiz de Cenzano et al. [26] for commercial baby foods with purees based on fruits and vegetables (5.4–15.4 µg Kg^{-1}) (these ones were not categorized as organic). Besides, these authors show that when other ingredients such as turkey or chicken (21.1–24.6 µg Kg^{-1}) or even fish (sole, sea bass or monkfish) (43.9–109 µg Kg^{-1}) [26] are included in the recipes, Se concentrations are higher. As a result of the scarce literature on Se concentration in baby foods, no conclusions can be drawn about whether the low Se contents found in our baby foods is due to organic ingredients or recipe composition (with little presence of animal – derived ingredients).

Regarding the bioaccessible concentration of Se, a greater uniformity of values was found, which ranged between 1.9 and 4.3 μg Kg^{-1} for VC and VV, respectively (Table 5). Khanam and Platel [16] have reported better Se bioaccessible results for cereal based composite meals (with many ingredients used in our recipes) which ranged between 27–33 μg Kg^{-1}. Bioaccessibility of Se from the organic weaning foods does not seem to be dependent on their total Se content. In fact, samples with the highest Se content containing meat ingredients such as PVC (24%), VC (15%), and VV (52%), presented lower bioaccessibility percentages than those with moderate Se content such as ZC (83%), SCF (100%), BPP (100%) and MFR (99%). These results are in agreement with a similar study in which bioaccessibility of Se was independent of the total content of this trace element in cereals, pulses and leafy green vegetables, making the information on Se bioaccessibility from foods relevant [27].

Despite the strong correlation between protein and Se contents, no significant correlation was found between Se solubility and protein content. Thus, the influence of protein sources over Se bioaccessibility is not clear. Some authors suggest that Se bioavailability decreases when the protein content in samples increases [28]. It seems that amino acids obtained from protein digestion increase the ionic strength of the aqueous phase on intestinal lumen where solubility of Se species decreases. On the contrary, Daniels [29] suggests that Se may be better absorbed from a high protein diet and Yan et al. [30] have reported a linear or log-linear dose-dependent increase between several Se – status indicators and dietary supplementation with protein isolates.

Fat content in weaning foods studied ranged between 0.65 and 3.12 g $100g^{-1}$ (Table 6). It has been indicated in a previous study that fat content of foods may impair Se bioaccessibility [17] mainly because Se species are poorly lipophilic molecules [28], and fat micelles formed during the digestion process may interfere with enzymes capacity to release Se present in peptide samples of low molecular weight [17]. Although, a negative significant correlation between both nutrients could not be established in the present study, (p= 0.065; r = -0.636), a decrease in Se bioaccessible concentration could be observed in samples with the highest fat content such as VC or VVC.

On the other hand, total dietary fiber contents ranged between 0.47 and 2.11 g $100g^{-1}$. These contents are in agreement with previous studies [31], where around 65% corresponded to the insoluble fraction and the remaining 35% to the soluble fraction. Although dietary fiber has traditionally had a negative role on minerals and trace elements´ bioaccessibility, currently there is little objective evidence that dietary fiber *per se* does not have this adverse effect on mineral metabolism [32]. According to our data, no statistically significant correlation was found between total fiber content and Se bioaccessibility in the simples studied. Indeed, some of the samples with the highest total fiber content such as VCP or SCF also presented moderately high amounts of bioaccessible Se (see Tables 5 and 6). Similarly, Baye et al. [33] found through *in vitro* studies that binding properties of some soluble fiber fractions such as gums or pectins are minimal; and even Sakai et al. [34], reported enhancing properties upon trace element absorption for fructo-oligosaccharides, inulin and pectin in rats. In the present study, a neutral effect of fiber could be observed upon Se bioaccessibility, both for soluble fiber and for the insoluble fiber. Therefore, a negative role on mineral absorption of weaning foods cannot be attributed to this nutritional component.

Table 5
Content of Se and Cd in the organic weaning food studied (mean ± standard deviation).

Sample	Se total (μg/Kg)	Se soluble (μg/Kg)	Cd total (μg/Kg)	Cd soluble (μg/Kg)
ZC	2.47 ± 0.27	2.04 ± 0.22	1.23 ± 0.75	0.17 ± 0.11
VVC	2.56 ± 0.43	1.96 ± 0.13	1.27 ± 0.34	0.18 ± 0.06
SCF	2.44 ± 0.22	2.91 ± 0.46	2.20 ± 0.32	0.22 ± 0.08
BAO	5.26 ± 0.81	3.97 ± 1.90	2.65 ± 0.23	0.45 ± 0.09
BPP	3.31 ± 1.59	3.41 ± 1.22	2.31 ± 0.57	1.23 ± 0.60
MFR	2.46 ± 0.62	2.44 ± 0.47	2.46 ± 0.74	1.38 ± 0.48
PVC	15.4 ± 1.4	3.76 ± 0.27	3.04 ± 0.50	0.28 ± 0.16
VC	12.8 ± 1.9	1.90 ± 0.81	1.56 ± 0.06	0.22 ± 0.08
VCP	3.70 ± 1.48	3.25 ± 0.24	3.64 ± 0.49	0.23 ± 0.12
VV	8.40 ± 1.11	4.35 ± 1.35	1.59 ± 1.43	0.24 ± 0.06

3.2. Cadmium

A recent report commissioned by the European Parliament points out that organic food production restricts the use of pesticides and antibiotics in farmed animals and results in lower concentrations of crop Cd [35]. Exposure to these compounds during pregnancy is associated with negative effects on intelligence and neuro-behavioral development. The greatest hazard of Cd in infancy is related to its capacity to affect the developing nervous system and bone formation [12,36]. In the organic weaning foods studied, Cd content ranged between 1.23 μg Kg^{-1} for ZC and 3.64 μg Kg^{-1} for VCP (see Table 5). These concentrations are considerably lower than those reported in Swedish weaning formulas (1.10–23.5 μg Kg^{-1}) [14] and Pakistani weaning foods (72.2 μg Kg^{-1}) [37], which were not categorized as organic. In fact, Cd content in our organic weaning foods may be considered similar to those reported in some studies with breast milk (mean levels around 2 μg L^{-1}) [38,39]. Nevertheless, these Cd levels in breast milk can be even lower (0.13 μg Kg^{-1}) when the study is performed amongst non – smoking women belonging to rural areas of Bangladesh, with a minimum industrial and traffic pollution [11]. As far as we know, there are few studies conducted to evaluate bioaccessibility and total Cd content in weaning foods and at the moment it is difficult to establish strong conclusions, but it seems using organic ingredients in the formulation of weaning foods could lead to reduced concentrations of this heavy metal.

Cd bioaccessibility of organic weaning foods studied ranged between 0.17 µg Kg^{-1} for ZC and 1.38 µg Kg^{-1} for MFR (Table 5). Throughout all the samples studied solubility was around 20% from the initial Cd concentration. As has already been mentioned, studies focusing on Cd bioaccessibility of infant food are scarce. Nevertheless, these values are lower than those of cooked food matrix such as vegetables (34–64%) [18], green beans (66%), carrots (73%), leek (76%) [40] and rice (70–74%) [41]. Many of these aforementioned ingredients are commonly used in weaning food recipes. Thus, these results, in addition to the initially smaller Cd concentration found in the analyzed samples, could show the benefit of using ingredients classified as organic to make up these kinds of foods.

Protein and dietary fiber contents did not have any statistically significant influence upon Cd bioaccessibility. Nevertheless, a negative statistical correlation was found between fat content and Cd bioaccesible concentration ($p < 0.05$; $r = -0.756$) for all samples studied. As has been previously mentioned regarding Se, this interaction can be justified by explaining that fat decreases the rate of enzymatic digestion, which also decreases Cd release from the molecules to which it is bound, given this, its bioaccessibility is also diminished.

Similarly, a negative statistical correlation was found ($p < 0.05$; $r = -0.777$) between Cd total content and Se bioaccessible concentration in all the studied samples. Respectively, a similar negative interaction was found between Se total content and Cd bioaccessible concentration; however, in this case it was not statistically significant ($p = 0.471$; $r = -0.277$). These results strengthen the protective role of Se upon heavy metals toxicity such as Cd. Interaction Se – Cd has been previously described in literature with animal models [42,43]. A study conducted with broilers have shown that diets with 0.3 mg Kg^{-1} of organic Se (mainly selenomethionine as Se –yeast) can help against the negative effects of moderate Cd levels in these diets (10 mg Kg^{-1}), but cannot counteract the negative effects of higher doses (100 mg Kg^{-1}) [44]. Likewise, Marval – León et al. [17] have also reported a significant negative correlation between Se bioaccessible and Cd content in several fish species. Similarly, the addition of organic Se in increasing concentrations (from 0.15 to 3 mg Kg^{-1}) could reduce the tissue deposition of Cd concentrations because the bioavailability of both elements decreases [45]. The mechanism which could explain this negative interaction between both elements consists of the formation of complexes or salts, which are either insoluble or poorly soluble in water. However, this will also depend on the chemical form in which Se is present, as well as the Se/Cd ratio [17]. Thus, in opposition to the protective effect of selenomethione discussed above, others previous studies with inorganic Se have shown that 0.1 mg Kg^{-1} Se as sodium selenite, added to diets contained 200 mg Kg^{-1} of Cd as cadmium chloride did not induce any significant changes in the levels of Cd accumulation in rats' kidney, liver [46]. However, another study [47] showed that sodium selenite supplementation in equimolar doses with cadmium chloride (8 µmol during 5–9 days) can decrease Cd retention in the tissues of suckling rats (mainly liver and kidney).

Table 6
Content of fiber (total and soluble), protein and fat (fresh matter) in the organic weaning food studied (mean ± standard deviation).

Sample	Total fiber (g /100 g)	Soluble fiber (g /100 g)	Protein (g /100 g)	Fat (g /100 g)
ZC	0.90 ± 0.03	0.58 ± 0.04	0.34 ± 0.01	2.46 ± 0.13
VVC	0.67 ± 0.05	0.44 ± 0.03	1.40 ± 0.04	3.12 ± 0.80
SCF	1.60 ± 0.47	1.32 ± 0.16	1.13 ± 0.53	2.76 ± 0.33
BAO	0.47 ± 0.06	0.30 ± 0.14	0.95 ± 0.06	0.65 ± 0.19
BPP	0.92 ± 0.08	0.69 ± 0.17	0.35 ± 0.07	1.02 ± 0.10
MFR	1.13 ± 0.05	0.66 ± 0.05	0.34 ± 0.05	0.87 ± 0.13
PVC	0.75 ± 0.07	0.38 ± 0.09	3.39 ± 0.24	2.36 ± 0.14
VC	0.78 ± 0.02	0.69 ± 0.05	3.24 ± 0.30	3.11 ± 0.25
VCP	2.11 ± 0.02	1.30 ± 0.09	2.75 ± 0.22	1.98 ± 0.19
VV	0.87 ± 0.10	0.58 ± 0.03	2.37 ± 0.17	2.47 ± 0.15

3.3. Probabilistic assessment

As already mentioned in Materials and methods section, a probabilistic model approach was developed to estimate the intake level of Se and Cd, which derived from consumption of 150–200 g (one jar) of these organic weaning foods. Dietary reference intakes (DRI) for the

Spanish population of 1–3 years old were considered for Se (20 µg/day) [48]. In the case of Cd, considering that it is a heavy metal, provisional tolerable weekly intake (PTWI) of 2.5 µg/kg body weight · week was used [49]. The models were developed from values of total and bioaccessible (soluble) trace element content. It should also be noted that the present statistical tool was completed using the variability of inorganic elements present in food as well as the variability of the organic weaning foods ingested. Both aspects determine the total amount of inorganic elements ingested.

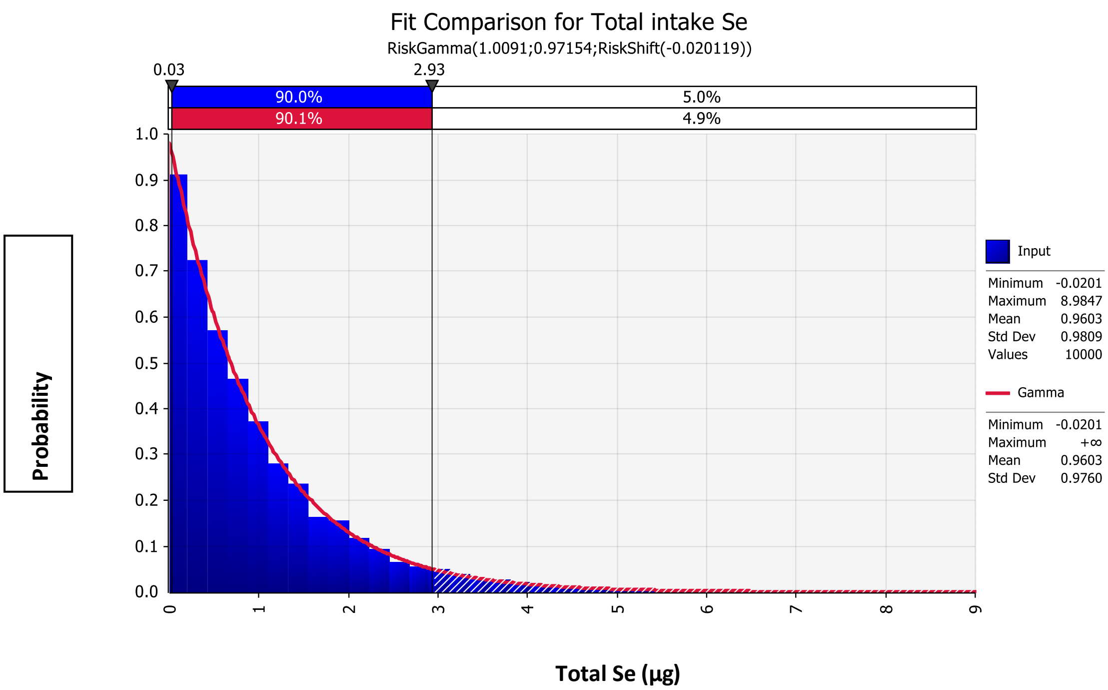
Gamma distribution showed the best fit to total concentration intakes of Se (AIC = 19609.14; Chi-Square = 60.00) (Fig. 1a). The mean Se intake estimated based on the aforementioned consumption pattern was of 0.96 µg with values of 0.66 µg and 2.91 for 50th and 95th percentiles, respectively. Thus, results derived from the simulation of the probabilistic model indicated that the intake level of Se through the consumption of one jar of these organic weaning foods would be below 2.91 µg for the 95% of infant population (15% of DRI for Se). There would even be individuals who do not exceed an intake above 0.03 µg (5th percentile) of Se from the consumption of these ecological weaning foods. Regarding the bioaccessible Se concentration data, a log normal distribution presented the best fit (AIC = -1685.18; Chi-Square = 74.36) (Fig. 1b). Estimated intakes were not above 0.52 µg and 1.00 µg for the 50th and 95th percentiles, respectively. In this later case, contributions to DRI of Se for the infant population group would not exceed 5%.

Considering the importance of Se and its important role in antioxidant selenoproteins for protection against oxidative stress, it can be said that the analysed organic jars did not represent a significant source for this inorganic micronutrient, making the search for alternative dietary sources necessary or even the formulation of these weaning foods with improved recipes with a high proportion of animal ingredients (meat or fish).

In the case of Cd, being a heavy metal element, the main objective is to know if the PWTI would be exceeded through the intake of these weaning foods (25–40 μg/week). This PWTI value range was obtained from multiplying 2.5 μg/kg body weight · week by the average body weight relevant for Spanish infants (10, 13 and 16 Kg for toddlers of 1, 2 and 3 years old respectively). The results derived from the simulation of the probabilistic models indicated that the exposure levels of Cd were low, being below the established PTWI. A BetaGeneral distribution was fitted to Cd total intake concentration data (AIC = −5771.05; Chi-Square = 274.91) (Fig. 2a). The mean Cd intake estimated was 0.34 μg and it did not exceed 0.69 μg for the 95th percentile of the studied population. This means that in the worst-case scenario, infants consuming a single serving of these weaning foods (equivalent to one jar) would only reach between 2.7 – 1.7% of PTWI for Cd. Even the maximum value of this distribution would be 0.79 μg (3.1 – 2.0% of PTWI). A log normal distribution was fitted to the bioaccessible Cd concentration intake data (AIC = −37455.58, Chi-Square = 72.34). In this case, the intakes of bioaccessible Cd per jar of ecological weaning foods would not exceed 0.22 μg for the 95th percentile (about 0.88–0.55% of PTWI).

Although there are some studies in the literature which have determined the concentration of Cd present in infant formulas [50,14] and human milk [51,38], few studies have been conducted to assess the risk of Cd exposure in infants through the consumption of weaning foods. There is just one study conducted by Eklund and Oskarsson [14] in infant formulas and weaning foods reporting an average total Cd intake of 0.44 μg / Kg day. Although these concentrations do not pose any risk to human health, the use of organic ingredients such as those analysed in our study would decrease in at least three times the risk. This is a remarkable effect considering the high toxicity of Cd on a population group as vulnerable as the infant population.

a)



b)

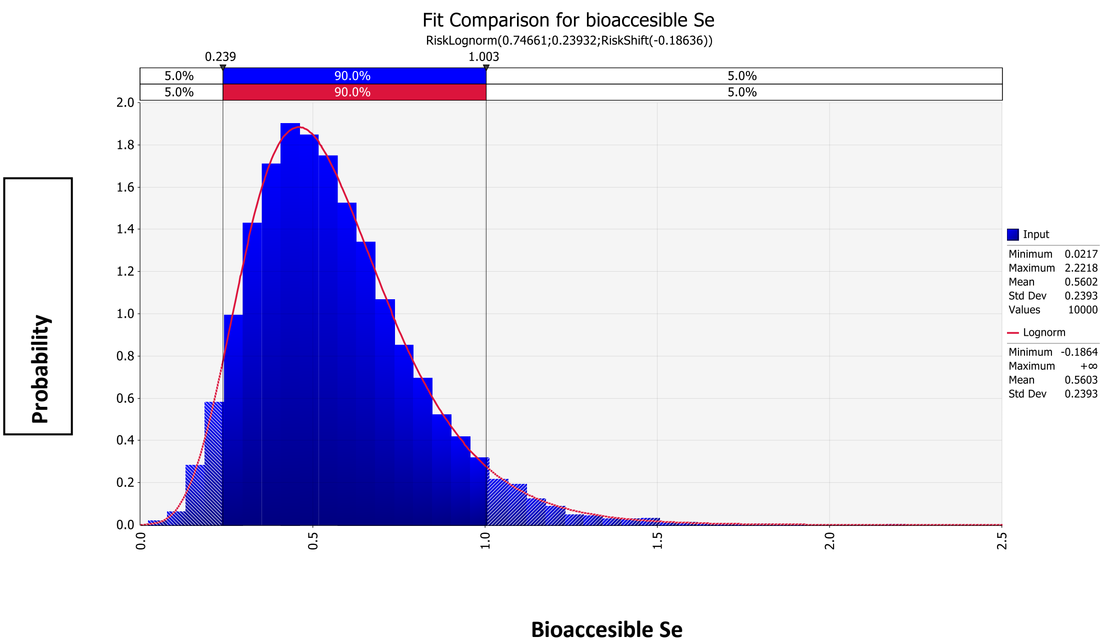
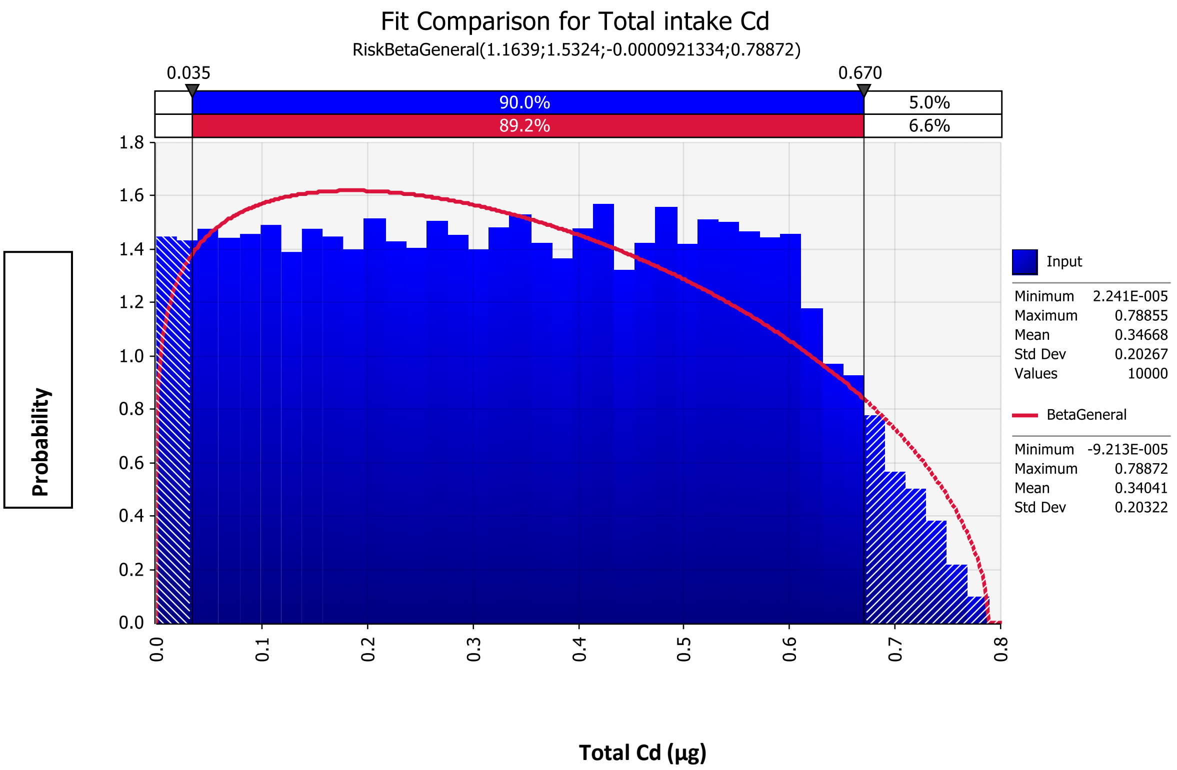


Fig. 1. Simulated data and fitted probabilistic distribution for Se total trace and bioaccessible

a)



b)

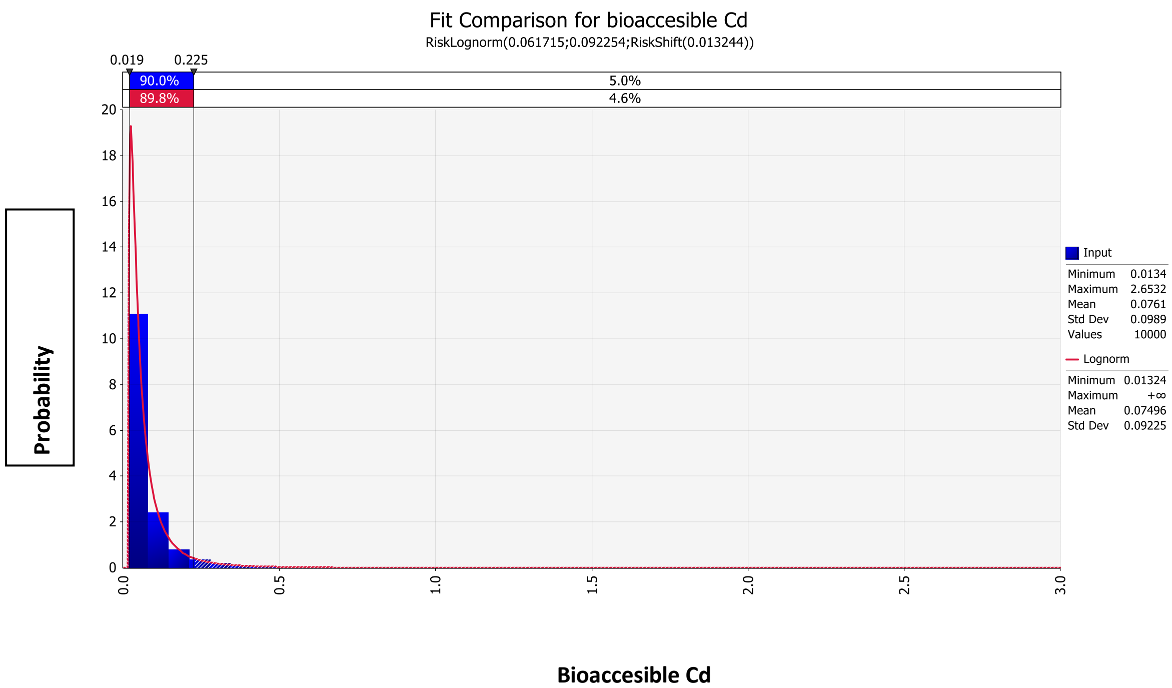


Fig. 2. Simulated data and fitted probabilistic distribution for Cd total trace and bioaccessible

4. Conclusions

Weaning foods analyzed in the present study showed extremely low Cd concentrations, with values that do not reach 3% of tolerable weekly intake in the worst case. This reveals that using organic ingredients in the formulation of recipes of baby foods could be one of the necessary commitments to reduce the presence of this heavy metal in diet. On the other hand, the analysed organic jars did not represent a significant source of Se. The probabilistic assessment developed, showed that contributions to DRI of Se for infants 1–3 years old by consumption of these weaning foods, are excessively low (15% at best). No clear evidence can be established that this is due to the presence of organic ingredients. However, considering the importance of this micronutrient as one of the main antioxidant in human nutrition, strategies aimed at increasing the content and bioaccessibility of this element in these infant foods should be encouraged. Finally, a negative interaction with positive effects between fat content and Cd bioaccessibility was observed in weaning foods. An antagonistic and equal effect between Se and Cd was also observed. These findings reinforce the protective role of Se against heavy metal toxicity.

Declaration of Competing Interest

The authors declare that they have no competing interests.

References

[1] E. Rembiałkowska, Organic food: effect on nutrient composition, in: B. Caballero, P. Finglas, F. Toldrá (Eds.), Encyclopedia of Food and Health, 1st ed., Academic Press – Elsevier, Oxford (GB), 2016, pp. 178–180.

[2] G.E. Lester, J.A. Manthey, B.S. Buslig, Organic vs conventionally grown Rio Red whole grapefruit and juice: comparison of production inputs, market quality, consumer acceptance, and human health-bioactive compounds, J. Agric. Food Chem. 55 (2007) 4474–4480.

[3] E. Koh, K. Wimalasiri, E. Renaud, A.E. Mitchell, A comparison of flavonoids, carotenoids and vitamin C in commercial organic and conventional marinara pasta sauce, J. Sci. Food Agric. 88 (2008) 344–354.

[4] R.F. Burk, O.A. Levander, M.E. Shils, J.A. Olson, M. Shike, Selenium, in: A.C. Ross (Ed.), Modern Nutrition in Health and Disease, 11th ed., Williams and Wilkins, Baltimore (USA), 1999, pp. 265–276.

[5] Z. Madeja, J. Sroka, C. Nyström, L. Björkhem Bergman, T. Nordman, A. Damdimopoulos, I. Nalvarte, L.C. Eriksson, G. Spyrou, J.M. Olsson,

M. Björnstedt, The role of thioredoxin reductase activity in selenium – induced cytotoxicity, Biochem. Pharmacol. 69 (12) (2005) 1765–1772.

[6] M.K. Anke, Essential and toxic effects of macro, trace, and ultratrace elements in the nutrition of animals, in: E. Merian, M. Anke, M. Ihnat, M. Stoeppler (Eds.), Elements and Their Compounds in the Environment, 1st ed., Wiley – VCH, Weinheim (Germany), 2004, p. 317.

[7] H. Guvenc, F. Karatas, M. Guvenc, S. Kunc, A.D. Aygun, S. Bektas, Low levels of selenium in mothers and their newborns in pregnancies with a neural tube defect, Pediatrics 95 (1995) 879–882.

[8] K. Polanska, A. Krol, W. Sobala, J. Gromadzinska, R. Brodzka, G. Calamandrei, F. Chiarotti, W. Wasowicz, W. Hanke, Selenium status during pregnancy and child psychomotor development-Polish Mother and Child. Cohort study, Pediatr. Res. 79 (2016) 863–869.

[9] H.M. Skroder, J.D. Hamadani, F. Tofail, L.A. Persson, M.E. Vahter, M.J. Kippler, Selenium status in pregnancy influences children's cognitive function at 1.5 years of age, Clin. Nutr. 34 (2015) 923–930.

[10] F. Cámara Martos, J.R. Marval León, R. Moreno Rojas, Selenium bioavilability in foods: the role of speciation and interaction with other dietary components, in: W. Morrison (Ed.), Selenium: Dietary Sources, Properties and Role in Human Health, 1st ed., Nova Science Publishers, New York (USA), 2015, pp. 1–28.

[11] M. Kippler, M.B. Hossain, C. Lindh, S.E. Moore, I. Kabir, M. Vahter, K. Broberg, Early life low-level cadmium exposure is positively associated with increased oxidative stress, Environ. Res. 112 (2012) 164–170.

[12] M. Sughis, J. Penders, V. Haufroid, B. Nemery, T.S. Nawrot, Bone resorption and environmental exposure to cadmium in children: a cross–sectional study, Environ. Health 10 (2011) 104.

[13] M. Rodríguez-Barranco, M. Lacasaña, C. Aguilar-Garduño, J. Alguacil, F. Gil, B. González-Alzaga, A. Rojas-García, Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis, Sci. Total Environ. 454–455 (2013) 562–577.

[14] G. Eklund, A. Oskarson, Exposure of cadmium from infant formulas and weaning foods, Food Addit. Contam. 16 (12) (1999) 509–519.

[15] W.P. Carvalho Santos, N. Moura Ribeiro, D.C. Muniz Batista Santos, M.G. Andrade Korn, M. Vieira Lopes, Bioaccessibility assessment of toxic and essential elements in produced pulses, Bahia, Brazil, Food Chem 240 (2018) 112–122.

[16] A. Khanam, K. Platel, Influence of domestic processing on the bioaccessibility of selenium from selected food grains and composite meals, J. Food Sci. Technol. 53 (3) (2016) 1634–1639.

[17] J.R. Marval-León, F. Cámara-Martos, M.A. Amaro-López, R. Moreno-Rojas, Bioaccessibility and content of Se in fish and shellfish widely consumed in Mediterranean countries: influence of proteins, fat and heavy metals, Int. J. Food Sci. Nutr. 65 (6) (2014) 678–685.

[18] P. Zhuang, Y. Li, F. Su, C. Zhang, H. Mo, Z. Li, Oral bioaccessibility and human exposure assessment of cadmium and lead in market vegetables in the Pearl River Delta, South China, Environ. Sci. Pollut. Res. 23 (2016) 24402–24410.

[19] J.C. Amiard, C. Amiard-Triquet, L. Charbonnier, A. Mesnil, P.S. Rainbow, W.X. Wang, Bioaccessibility of essential and non-essential metals in commercial shellfish from Western Europe and Asia, Food Chem. Toxicol. 46 (2008) 2010–2022.

[20] A.L. Maulvault, R. Machado, C. Alfonso, H.M. Lourenço, M.L. Nunes, I. Coelho, T. Langerholc, A. Marques, Bioaccessibility of Hg, Cd and As in cooked black scabbard fish and edible crab, Food Chem. Toxicol. 49 (11) (2011) 2808–2815.

[21] F. Cámara, M.A. Amaro, R. Barberá, G. Clemente, Bioaccessibility of minerals in school meals: comparison between dialysis and solubility methods, Food Chem. 92 (3) (2005) 481–489.

[22] J.R. Marval León, F. Cámara Martos, F. Pérez Rodríguez, R. Moreno Rojas, Optimization of Selenium determination based on the HG-ET-AAS method for its application to different food matrices, Food Anal. Methods 5 (5) (2012) 1054–1061.

[23] AOAC International, Official Methods of Analysis of AOAC International, 18th ed., AOAC International, Gaithersburg (MD), 2005.

[24] M. Roman, Selenium: properties and determination, in: B. Caballero, P. Finglas, F. Toldrá (Eds.), Encyclopedia of Food and Health, 1st ed., Academic Press – Elsevier, Oxford (GB), 2016, pp. 178–180.

[25] N. Zand, B.Z. Chowdhry, F.B. Zotor, D.S. Wray, P. Amuna, F.S. Pullen, Essential and trace elements content of commercial infant foods in the UK, Food Chem. 128 (2011) 123–128.

[26] M. Ruiz de Cenzano, A. Rochina Marco, M.L. Cervera, M. de la Guardia, Evaluation of the content of antimony, arsenic, bismuth, selenium, tellurium and their in- organic forms in commercially baby foods, Biol. Trace Elem. Res. 180 (2) (2017) 355–365.

[27] A. Khanam, K. Platel, Bioaccessibility of selenium, selenomethionine and seleno-cysteine from foods and influence of heat processing on the same, Food Chem. 194 (2016) 1293–1299.

[28] J. Moreda-Piñeiro, A. Moreda-Piñeiro, V. Romarís-Hortas, R. Domínguez-González, E. Alonso-Rodríguez, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, P. Bermejo-Barrera, ICP-MS for the determination of selenium bioavailability from seafood and effect of major food constituents, Microchem. J. 108 (2013) 174–179.

[29] L.A. Daniels, Selenium metabolism and bioavailability, Biol. Trace Elem. Res. 54 (1996) 185–199.

[30] L. Yan, P.G. Reeves, L.K. Johnson, Assessment of selenium bioavailability from naturally produced high – selenium soy foods in selenium – deficient rats, J. Trace Elem. Med. Biol. 24 (4) (2010) 223–229.

[31] L. Davidsson, J. Mackenzie, P. Kastenmayer, A. Rose, B.E. Golden, P.J. Agget, R.F. Hurrell, Dietary fiber in weaning cereals: a study of the effect on stool characteristics and absorption of energy, nitrogen, and minerals in healthy infants, J. Pediatr. Gastroenterol. Nutr. 22 (2) (1996) 167–179.

[32] I.T. Johnson, Dietary fiber: physiological effects, in: B. Caballero, P. Finglas, F. Toldrá (Eds.), Encyclopedia of Food and Health, 1st ed., Academic Press – Elsevier, Oxford (GB), 2016, pp. 400–403.

[33] K. Baye, J.P. Guyot, C. Mouquet-Rivier, The unresolved role of dietary fibers on mineral absorption, Crit. Rev. Food Sci. Nutr. 57 (5) (2017) 949–957.

[34] K. Sakai, A. Ohta, K. Shiga, M. Takasaki, T. Tokunaga, H. Hara, The cecum and dietary short-chain fructooligosaccharides are involved in preventing post-gastrectomy anemia in rats, J. Nutr. 130 (6) (2000) 1608–1612.

[35] The organic food: panacea for health? Lancet 389 (2017) 672.

[36] L. Järup, Hazards of heavy metal contamination, Br. Med. Bull. 68 (2003) 167–182.

[37] T.G. Kazi, N. Jalbani, J.A. Baig, M.B. Arain, H.I. Afridi, M.K. Jamali, A.Q. Shah, A.N. Memon, Evaluation of toxic elements in baby foods commercially available in Pakistan, Food Chem. 119 (4) (2010) 1313–1317.

[38] F. Maciel Rebelo, E. Dutra Caldas, Arsenic, lead, mercury and cadmium: Toxicity, levels in breast milk and the risks for breastfed infants, Environ. Res. 151 (2016) 671–688.

[39] A. Winiarska Mieczan, Cadmium, lead, copper and zinc in breast milk in Poland, Biol. Trace Elem. Res. 157 (2014) 36–44.

[40] A. Pelfrene, C. Waterlot, A. Guerin, N. Proix, A. Richard, F. Douay, Use of an in vitro digestion method to estimate human bioaccessibility of Cd in vegetables grown in smelter-impacted soils: the influence of cooking, Environ. Geochem. Health 37 (2015) 767–778.

[41] P. Zhuang, C. Zhang, Y. Li, B. Zou, H. Mo, K. Wu, J. Wu, Z. Li, Assessment of influences of cooking on cadmium and arsenic bioaccessibility in rice, using an in vitro physiologically-based extraction test, Food Chem. 213 (2016) 206–214.

[42] X. Hu, D. Chandler, J. Fernandes, M. Orr, L. Hao, K. Uppal, D.C. Neujar, D.P. Jones, Y.M. Go, Selenium supplementation prevents metabolic and transcriptomic responses to cadmium in mouse lung, Biochim. Biophys. Acta 1862 (2018) 2417–2426.

[43] R. Zhang, Y. Liu, L. Xing, N. Zhao, Q. Zheng, J. Li, J. Bao, The protective role of selenium against cadmium-induced hepatotoxicity in laying hens: expression of Hsps and inflammation-related genes and modulation of elements homeostasis, Ecotoxicol. Environ. Saf. 159 (2018) 205–212.

[44] A. Al-Wsrli, E. Zoidis, A.C. Pappas, N. Demiris, G. Zervas, K. Fegeros, The role of organic selenium in cadmim toxicity: effects on broiler performance and health status, Animal 7 (2013) 386–393.

[45] A.C. Pappas, E. Zoidis, C.A. Georgiou, N. Demiris, P.F. Surai, K. Fegeros, Influence of organic selenium supplementation on the accumulation of toxic and essential trace elements involved in the antioxidant system of chicken, Food Addit. Contam. Part A 28 (2011) 446–454.

[46] E.H. Jinhen, I. Messaoudi, H. Fatima, A. Kerkeni, Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver and kidney of the rat: histology and Cd accumulation, Food Chem. Toxicol. 46 (2008) 3522–3527.

[47] M. Lazarus, T. Orct, J. Jurasoviae, M. Blanusa, The effect of dietary selenium supplementation on cadmium absorption and retention in suckling rats, Biometals 22 (2009) 973–983.

[48] FESNAD, Ingestas Dietéticas de Referencia (IDR) para la Población Española, 2010, Act Diet. 14 (4) (2010) 196–197.

[49] European Food Safety Authority – EFSA, Cadmium in food. Scientific Opinion of the Panel on Contaminants in the Food Chain, (2009) https://www.efsa.europa.eu/en/efsajournal/pub/980.

[50] S. Dubascoux, M. Nicolas, C.F. Rime, J.R. Payot, E. Poitevin, Simultaneous determination of 10 ultratrace elements in infant formula, adult nutritionals and milk products by ICP/MS after pressure digestion: single-laboratory validation, J. AOAC Int. 98 (4) (2015) 953–961.

[51] M. Bassil, F. Daou, H. Hassan, O. Yamani, J.A. Kharma, Z. Attieh, J. Elaridi, Lead, cadmium and arsenic in human milk and their socio-demographic and lifestyle determinants in Lebanon, Chemosphere 191 (2018) 911–921.

Conclusiones




4. CONCLUSIONES

PRIMERA. Las semillas de guisantes y lentejas se encuentran dentro de un amplio grupo de alimentos, las leguminosas. Gracias a su bajo coste económico y a su alto valor nutricional se destinan tanto a la alimentación humana como a la animal. No obstante, también contienen otras sustancias como inhibidores enzimáticos, oxalatos, fitatos y polifenoles que pueden reducir su calidad nutricional. A pesar de ello, se han investigado diferentes metodologías (procesos de pelado, remojo, germinación, fermentación y cocción y el uso de ciertas enzimas) para reducir e incluso eliminar estos factores antinutricionales (Capítulo 1).

SEGUNDA. Las leguminosas son una buena fuente dietética de algunos elementos inorgánicos como hierro, zinc, cobre y manganeso. No son sin embargo, buenas fuentes dietéticas para calcio y magnesio, tanto desde el punto de vista de su contenido total como bioaccesible. Como consecuencia de lo primero, su consumo debe fomentarse e incrementarse como parte de una dieta saludable, considerando además su bajo contenido en grasa y su alto contenido en proteínas de origen vegetal (Capítulo 2).

TERCERA. El cocinado de las leguminosas conlleva una disminución en la concentración de los elementos inorgánicos inicialmente presentes en la legumbre cruda, probablemente a una solubilización de éstos en el agua de cocción. No obstante, la bioaccesibilidad de los elementos inorgánicos en las legumbres cocinadas se incrementa frente a las crudas como consecuencia de la destrucción de algunos componentes nutricionales presentes en la legumbre cruda (Capítulo 2).

CUARTA. La concentración total y bioaccesible de los elementos inorgánicos analizados en las formulaciones de los potitos ecológicos, fueron inferiores a las reportadas en la bibliografía para recetas con ingredientes similares pero no categorizadas con el atributo de “ecológico”. Esto puede llegar a ser un motivo de preocupación, si como en el caso de nuestro estudio las contribuciones a las Ingestas Dietéticas de Referencia no superan el 5% para algunos oligoelementos como calcio, hierro y zinc, indispensables en la época de crecimiento (Capítulo 3).

QUINTA. La presencia de proteínas, tanto animales como vegetales, en las recetas de potitos estudiadas incrementa la bioaccesibilidad de hierro y en menor extensión la bioaccesibilidad de zinc. Junto a esto, la vitamina C también puede promover la bioaccesibilidad de hierro cuando la concentración proteica del alimento es baja (Capítulo 3).

SEXTA. Los potitos ecológicos presentaron contenidos de cadmio extremadamente bajos e inferiores a los reportados en la literatura para formulaciones convencionales. Esto revela que la utilización de ingredientes ecológicos podría ser uno de los pasos para reducir la presencia de este metal pesado en la dieta. No obstante, las formulaciones convencionales tampoco suponen un riesgo para la salud de los infantes, contribuyendo con porcentajes muy bajos a la PTWI de este elemento (Capítulo 4).

SÉPTIMA. Al igual que ocurre para el resto de micronutrientes inorgánicos las concentraciones de selenio analizadas en los potitos ecológicos fueron bajas, con concentraciones muy pequeñas a la Ingesta Dietética de Referencia de este micronutriente esencial. Se observa igualmente una interacción negativa entre selenio y cadmio en los potitos ecológicos analizados lo que refuerza el papel de este elemento frente a la toxicidad por metales pesados (Capítulo 4).